Neurochemical and cognitive aftermaths of surgery

Studies on short- and long-term effects of surgery and anesthesia

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Gothenburg, Sweden, 2020

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Cover illustration: Mattias Danielson

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ISBN 978-91-7833-994-5 (PRINT)
ISBN 978-91-7833-995-2 (PDF)
http://hdl.handle.net/2077/65129

Printed in Borås, Sweden 2020
Printed by Stema Specialtryck AB

"Science is the poetry of reality."

Richard Dawkins

Abstract

Background: Each year, around the world, more than 230 million patients have surgery. Improvements in healthcare have resulted in older and sicker patients undergoing surgical interventions. As a result, surgical safety has become a global public-health concern. Cognitive impairment has emerged as the most common postoperative complication in these older individuals. The etiology of this condition is unknown, although episodes of hypooxygenation/hypoperfusion, negative impacts of anesthetic drugs, cerebral microemboli from cardiopulmonary bypass during cardiac surgery, and neuroinflammation have been implicated.

Aims: To explore the mechanisms behind this postoperative cognitive dysfunction, we focused on the impacts of surgery on: 1) blood-brain barrier (BBB) function; 2) changes in the levels of systemic and neuroinflammatory biomarkers; and 3) biochemical evidence of perioperative neuronal damage. We also evaluated whether an unfavorable late neurocognitive postoperative outcome was associated with an enhanced neuroinflammatory response or cerebral biomarker evidence of neuronal injury. Finally, we investigated whether a single perioperative dose of methylprednisolone attenuates the postoperative BBB dysfunction and prevents neuroinflammation after cardiac surgery.

Methods: We conducted a prospective, observational, two-center study with patients who were undergoing elective major orthopedic surgery. Cognitive function was evaluated preoperatively, at discharge, and at 3 months postoperatively. Biochemical markers of inflammation, neuronal damage, brain amyloidosis, and BBB function were measured in cerebrospinal fluid (CSF) and blood samples during the initial 48 hours postoperatively. Furthermore, in a prospective, randomized, double-blinded, double-armed study, 30 patients who were undergoing elective open heart surgery were randomized to a single dose of either methylprednisolone or placebo. CSF and blood samples obtained preoperatively and at 24 hours after surgery were analyzed for biochemical markers of inflammation, neuronal damage, and BBB function.

Results: Disruption of BBB function, manifested as an increased CSF to serum albumin quotient, was detected after both cardiac and orthopedic surgeries. Both orthopedic and cardiac surgeries were associated with a pronounced increase in inflammatory biomarkers in the CSF and blood. The CSF inflammatory biomarkers were significantly associated with long-term cognitive decline 3 months after orthopedic surgery.
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Surgery resulted in increased levels of biomarkers of neuronal damage and brain amyloidosis in the CSF and blood, although there was no association between these biomarkers and postoperative cognitive decline. None of preoperative biomarkers were predictive of postoperative cognitive decline. Methylprednisolone attenuated the systemic inflammatory reaction but not the BBB dysfunction that followed cardiac surgery.

Conclusions: Surgery induces a profound systemic inflammatory reaction, followed by disruption of the BBB. Single-dose treatment with a potent corticosteroid did not attenuate the BBB disruption. The postoperative neuroinflammatory reaction, assessed as biomarker levels in the CSF, is significantly associated with long-term cognitive decline, whereas the increased postoperative markers of neuronal damage showed no such association

Keywords: Anesthesia, blood-brain barrier, brain amyloidosis, cardiac surgery, orthopedic surgery, inflammation, neuroinflammation, neuronal damage, cognitive decline, postoperative cognitive dysfunction

Sammanfattning på svenska

I takt med industrialiseringen har det skett en demografisk förskjutning mot en allt äldre befolkning. Detta i kombination med de senaste decenniernas medicinska framsteg har medfört att det utförs successivt mer avancerade kirurgiska ingrepp på allt äldre patienter. Det har länge varit känt att det finns en risk för kognitiv nedsättning, dvs försämring av hjärnans högre funktioner, efter kirurgiska ingrepp hos framförallt äldre patienter. Hur omfattande problemet är har först uppenbarats på senare tid, när större studier har visat att ca 25% av patienterna har kognitiv nedsättning första veckan efter kirurgi och efter 3 månader har 10% kvarstående problem. Detta gör kognitiv nedsättning till den vanligaste komplikationen efter kirurgi. Mekanismerna bakom tillståndet är dock okända. Det har framförts teorier om att orsaken skulle vara långtidseffekter av anestesimedel, små blodproppar från hjärt-lungmaskinen till hjärnan vid hjärtkirurgi, påskyndande av demensutveckling, perioder med lågt blodtryck under operationen eller en inflammatorisk reaktion i hjärnan.

Vi har i dessa studier utforskat bakomliggande mekanismer, med fokus framförallt på en möjlig inflammatorisk orsak. I samband med kirurgi sker alltid cellskada och de ämnen som frisätts aktiverar vårt medfödda immunsystem vilket ger en lokal inflammatorisk reaktion som i sig är en viktig del i läkningsprocessen. Blod-hjärn barriären har som uppgift att strikt reglera vilka ämnen som passerar från blodet till centrala nervsystemet, där det i normalfallet är en mycket låg grad av immunologisk aktivitet. Inflammatoriska signalsubstanser som kommer ut i blodbanan efter kirurgi ger en påverkan på kroppens övriga organ och har visat sig, i experimentella studier, via en ökad genomsläpplighet i blod-hjärn barriären, kunna sprida inflammationen även till hjärnan.

Genom att undersöka hur nivåerna av olika substanser i blod och ryggmärgsvätska påverkas av kirurgi har vi studerat blod-hjärn barriärens funktion, tecken till nervcellsskada, påverkan på demensmarkörer och grad av inflammation. Vi har undersökt patienters kognitiva funktion före operationen, innan hemgång samt vid ett återbesök 3 månader efter det kirurgiska ingreppet. I ett försök att minska inflammation och påverkan på blod-hjärn barriären gavs patienter en engångsdos metylprednisolon (en inflammationsdämpande steroid) före hjärtoperation.

Vi fann en uttalad ökning av inflammatoriska markörer i blod följt av ökad genomsläpplighet i blod-hjärn barriären efter kirurgi. Under de första 48 timmarna efter ortopedisk kirurgi skilde sig sedan det inflammatoriska svaret i hjärnan tydligt mellan de patienter som efter tre månader kom att ha kvarstående kognitiv nedsättning och de som återhämtade funktionen. Metylprednisolon minskade inte påverkan på blod-hjärn barriären eller inflammationen i hjärnan.

I blod och ryggmärgsvätska ökade nivåerna av ämnen som tyder på nervcellsskada, men det fanns inget samband mellan nivåerna av dessa markörer och utebliven kognitiv återhämtning. Vi hittade ingen markör i proverna som togs före operationen som skulle kunna förutsäga patienternas kognitiva påverkan på kort eller lång sikt.

List of papers

This thesis is based on the following appended papers, which are referred to in the text by their assigned Roman numerals:

I. Danielson M, Wiklund A, Granath F, Blennow K, Mkrtchian S, Nellgard B, Oras J, Jonsson Fagerlund M, Granstrom A, Schening A, Rasmussen LS, Erlandsson Harris H, Zetterberg H, Ricksten SE, Eriksson LI

Neuroinflammatory markers associate with cognitive decline after major surgery: Findings of an explorative study

Ann Neurol. 2020 Mar;87(3):370-382.
DOI: 10.1002/ana.25678

II. Danielson M, Wiklund A, Granath F, Blennow K, Mkrtchian S, Nellgard B, Oras J, Jonsson Fagerlund M, Granstrom A, Schening A, Rasmussen LS, Erlandsson Harris H, Zetterberg H, Ricksten SE, Eriksson LI

The association between cerebrospinal fluid biomarkers of neuronal injury or brain amyloidosis and cognitive decline after major surgery

Accepted for publication
Br J Anaesth. 2020

III. Danielson M, Reinsfelt B, Westerlind A, Zetterberg H, Blennow K, Ricksten, SE

Effects of methylprednisolone on blood-brain barrier and cerebral inflammation in cardiac surgery-a randomized trial

J Neuroinflammation. 2018;15:283
DOI: 10.1186/s12974-018-1318-y

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Abbreviations

Aβ	β-amyloid protein
Aβ1-40	the 40-amino-acid isoform of β-amyloid
Aβ1-42	the 42-amino-acid isoform of β-amyloid
AD	Alzheimer's disease
ApoE	apolipoprotein E
APP	amyloid precursor protein
ANOVA	analysis of variance
ANCOVA	analysis of covariance
AS	aortic stenosis
ASA	American Society of Anesthesiologists
AVR	aortic valve replacement
BBB	blood-brain barrier
BSA	body surface area
CABG	coronary artery bypass grafting
CAM-ICU	the Confusion Assessment Method for the Intensive Care Unit
CNS	central nervous system
CO	cardiac output
CONSORT	consolidated standards of reporting trials
CPB	cardiopulmonary bypass
CSF	cerebrospinal fluid
DAMP	damage-associated molecular patterns
DSM-5	The Diagnostic and Statistical Manual of Mental Disorders, 5th edition
ECC	extracorporeal circulation
ECL	electro-chemiluminescence
ELISA	enzyme-linked immunosorbent assay
GA	general anesthesia
GFAP	glial fibrillary acidic protein
HMGB1	high mobility group box 1 protein
HR	heart rate
ICAM-1	intercellular adhesion molecule-1
ICD 10	International Statistical Classification of Diseases, 10th edition
ICU	intensive care unit
IL-X	interleukin-X
IQR	interquartile range
ISPOCD	International Study of Post-Operative Cognitive Dysfunction Group

LAM	leukocyte adhesion molecule
LPS	lipopolysaccharide
LVEF	left ventricular ejection fraction
MAP	mean arterial pressure
MMP	matrix metalloproteinase
MMSE	mini mental state exam
MRI	magnetic resonance imaging
NFL	neurofilament light chain
NFT	neurofibrillary tangle
NMDA	N-methyl-D-aspartate
NSE	neuron-specific enolase
NVU	neurovascular unit
NYHA	New York Heart Association
PACU	post-anesthesia care unit
PAMP	pathogen-associated molecular pattern
PC1-3	principal component 1-3
PCA	principal component analysis
PCR	polymerase chain reaction
PET	positron emission tomography
POCD	postoperative cognitive dysfunction
POD	postoperative delirium
PRR	pattern recognition receptors
p-Tau	phosphorylated-Tau protein
SAP	systolic arterial pressure
SAVR	surgical aortic valve replacement
SD	standard deviation
SEM	standard error of the mean
TIVA	total intravenous anesthesia
TJ	tight junction
TNF-α	tumor necrosis factor-α
t-Tau	total-Tau protein
VAS	visual analog scale

1. Introduction

1.1 Background

The mind is a precious, yet fragile, gift of evolution. For most of us, the deterioration of our mental abilities is a far more frightening prospect than debilitation of the rest of the body. Nevertheless, and even though reports of mental side-effects of surgery and anesthesia date back to the birth of modern surgery[1, 2], this subject remained for a long time largely unexplored.

1.1.1 "Pumpheads" in cardiac surgery

In the 1950's, the first heart-lung machines were introduced in clinical practice, making open-heart surgery feasible[3]. Over time, concerns were raised regarding the potential side-effects of the use of the heart-lung machine, as some patients exhibited impaired cognitive functions after otherwise uneventful surgery. This was confirmed in a number of studies, in which the incidence of cognitive impairment 1 week following surgery was found to be in the range of 50%–70%[4]. However, there was no consensus in the definition of cognitive deficit, which tests should be used, or the timing of the tests. By the late 1980's, the concept emerged of postoperative cognitive dysfunction (POCD), signifying a patient who did not return to their baseline cognition level after surgery. A systematic review revealed that 23% of patients experienced a cognitive deficit 2 months after surgery[5]. This was attributed to the use of cardiopulmonary bypass (CPB) in cardiac surgery, and cardiac surgery performed without the use of CPB (off-pump surgery) was suggested as a way to reduce this risk. Subsequent studies, however, failed to show a neuroprotective effect of using off-pump surgery[6].

1.1.2 The 1990's

The subject of POCD attracted renewed interest with a publication in 1998 from the International Study of Post-Operative Cognitive Dysfunction (ISPOCD) group. In this study, more than 1,000 patients who were undergoing different types of non-cardiac surgery were examined both before and after surgery using a comprehensive

test battery. Perioperative neurocognitive decline was detected in 26% of the patients 1 week after surgery, and in 10% of the patients 3 months after surgery[7]. These results suggested that POCD was a common complication, not only following cardiac surgery, but also after general surgery.

1.2 Perioperative neurologic complications

The broad spectrum of possible perioperative neurological complications is, for the sake of completeness, briefly discussed in this section. Thereafter, this thesis will focus on POCD.

Worldwide, more than 230 million patients undergo surgery annually, with the consequence that surgical safety has become a significant global public-health concern[8]. At the same time, we are facing an increasingly aging population. This, combined with more effective and safer anesthesia and surgery, has resulted in older and sicker patients being more likely than ever before to be exposed to surgical interventions. In the USA, 15% of the population are older than 65 years and they receive 35% of all in-patient surgeries[9].

Elderly patients have increased risks of postoperative complications and mortality[10]. These complications include the perioperative neurocognitive disorder of acute delirium and POCD. Delirium is now recognized as the most common complication following surgery in older adults, with an incidence of up to 50% [11]. It is associated with adverse outcomes, including prolonged hospitalization, loss of functional independence, impaired cognitive function, and death[12-14].

The occurrence of stroke after cardiac surgery significantly prolongs the postoperative hospital stay (11 versus 7 days), as well as the intensive care unit stay (3 versus 2 days)[15]. Furthermore, in-hospital mortality is significantly higher for patients who suffer a perioperative stroke, as compared to those who do not (14.4% versus 2.7%)[15].

Cognitive decline is associated with decreased quality of life 1 and 5 years after cardiac surgery[16, 17]. It is associated with increased mortality, risk of premature departure from the labor market, and dependency on social welfare payments after non-cardiac surgery[18].

1.2.1 Stroke

Stroke, which is a leading global cause of premature death and disability, is responsible for 6.2 million deaths annually. Since, treatment options are few, clinical efforts focus mainly on the prevention and treatment of stroke and the other sequelae of

cerebrovascular disease. In the perioperative setting, patients are at particular risk of stroke, with the highest incidence seen for cardiovascular surgery (range of 1.9–9.7% in different studies)[19], while in non-cardiovascular, non-neurological surgery the stroke incidence is in the range of 0.1–1.9%[20].

1.2.2 Delirium

Delirium is an acute and fluctuating alteration of the mental state of the subject, involving an altered level of consciousness and disturbance in attention (i.e. reduced ability to direct, focus, sustain, and shift attention). It is defined by either the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD 10) or by the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM- 5). Postoperative delirium (POD) occurs up to 5 days after surgery. Delirium takes a hypoactive (decreased alertness and motor activity) or hyperactive (agitated and combative) form. If not specifically looked for in the diagnostic workup, the hypoactive form often goes undetected[21].

The use of established diagnostic tools is recommended. The Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) has shown strong reliability and validity for assessing delirium[22]. The CAM-ICU evaluates mental status, inattention, disorganized thinking, and altered level of consciousness.

1.2.3 Postoperative cognitive dysfunction

Cognitive functions are mental processes that allow us to perform various tasks. The processes include learning, information processing, flexibility, problem solving, decision making, reasoning, remembering, and attention. POCD involves a decline of the cognitive abilities of the patient following a surgical procedure.

In contrast to delirium and dementia, there has been a lack of consensus as to how POCD should be defined. Consequently, there are major differences in methodology between the performed studies in terms of which test batteries are used, the interval between sessions, which endpoints are analyzed, and which statistical methods are applied. This makes comparisons of the studies challenging, and creates difficulties with respect to drawing definitive conclusions about various interventions or risk factors.

The previously mentioned ISPOCD-1 study introduced the concept of an age-matched control group to adjust for learning effects from repeated tests[7].
In 2018, an updated nomenclature for POCD was presented[23], which aimed to harmonize the criteria and nomenclature to other conditions of cognitive decline

from the DSM-5 and ICD-10. This results in a clinical nomenclature, with each category requiring symptoms or complaints from the patient or from relatives. Efforts to define perioperative neurocognitive disorder research criteria are underway, but not yet published.

1.2.4 Dementia

Dementia, which is a chronic disorder of the mental processes resulting from brain disease or injury and affecting cognitive domains such as memory, personality changes, and impaired reasoning, has a global prevalence of more than 50 million persons[24].

Alzheimer's disease (AD) is a fatal, progressive neurodegenerative disorder, accounting for the majority of dementia cases.

A recent case-control study based on the Swedish Twin Registry found no clear evidence for increased risk of dementia after exposure to surgery and anesthesia[25].

1.3 Risks, etiologies, and mechanisms of postoperative cognitive dysfunction

1.3.1 Risk factors

According to the results of the ISPOCD-1 study, increasing age, fewer years of formal education, longer duration of surgery, and complications (re-operation, infection, respiratory complications) are all factors that increase the risk of POCD at 1 week, while only age remains a risk factor for POCD 3 months after general surgery[7]. Furthermore, preoperative cognitive impairment is associated with an increased risk of POCD[26].

1.3.2 General anesthesia and surgery

To determine whether general anesthesia (GA) itself is a risk factor for POCD, several studies have compared patients who underwent surgical interventions that involved GA or non-GA methods, such as regional anesthesia or sedation.

In 2003, Rasmussen and colleagues enrolled 428 elderly patients and found no significant difference in the incidence of cognitive dysfunction 3 months after operations performed with either GA or regional anesthesia[27]. Repeated studies undertaken since then have failed to identify GA as a risk factor for POCD[28, 29].

A recent study described a significant increase in the levels of plasma biomarkers of neuronal injury after exposure to GA and surgery, suggesting that surgery under GA may induce neuronal injury[30]. However, in that study, postoperative cognitive performance was not measured.

Animal studies have shown how an isolated peripheral surgical trauma activates the innate immune system, releasing mediators that disrupt the integrity of the BBB [31], allowing the migration of macrophages into the central nervous system, resulting in neuroinflammation and subsequent cognitive impairment[32].

1.3.3 Anesthetic agents and depth of anesthesia

Several studies have evaluated whether the risk of POCD differs with GA using inhalational agents, as compared to GA with total intravenous anesthesia (TIVA). A recent meta-analysis has shown low-grade evidence for a risk-reducing effect of TIVA[33]. Further studies on this topic are underway. Depth of anesthesia is another proposed risk factor for POCD, although monitoring and adjusting the level of anesthesia has hitherto proven unsuccessful in terms of preventing POCD[34].

1.3.4 Cerebral perfusion and oxygenation

Cerebral hypoperfusion and hypooxygenation, which are possible consequences of intraoperative systemic hypotension and can lead to brain damage, were not identified as risk factors in the ISPOCD study[7]. An association between prolonged episodes of cerebral desaturation during cardiac surgery (measured with a non-invasive cerebral oximeter) and early cognitive decline has been demonstrated[35].

1.3.5 Genetics

The polymorphic apolipoprotein E (ApoE), which is a lipid-binding protein that is involved in the build-up of myelin sheets and cell membranes, has been implicated in the repair of neuronal injury. The gene that encodes ApoE exists in three allelic isoforms: ApoE-epsilon 2, 3 and 4. The isoform ApoE-epsilon 4 is strongly associated with AD[36]. A meta-analysis carried out in 2014 concluded that there is an association between the carrier status of the ApoE-epsilon 4 allele and the risk of POCD at 1 week postoperatively[37].

1.3.6 Cardiac surgery, use of heart-lung machine

CPB, which exposes the blood to foreign materials and carries the risk of solid or gaseous cerebral microemboli, has been considered a major risk factor, although studies have failed to show any difference in the incidence of POCD between on-pump and off-pump surgeries[6, 38].

To avoid gaseous microemboli after open chamber cardiac valve operation, many centers flood the open cardiac chamber with the more soluble gas carbon dioxide during the procedure. However, a randomized trial failed to find a protective effect of carbon dioxide usage on cognitive decline[39].

1.3.7 Alzheimer's disease pathology

Hallmark changes of neurochemical biomarkers in patients with AD include increased levels of cerebrospinal fluid (CSF) total-Tau (t-Tau), phosphorylated-Tau (p-Tau), neurofilament light chain (NFL), and decreased levels of amyloid β1-42 and amyloid β1-40 (Aβ1-42 and Aβ1-40).

Patients scheduled for CABG who subsequently developed POCD had preoperatively significantly lower plasma levels of Aβ1-42 and Aβ1-40[40]. Patients undergoing total hip or knee replacement in spinal anesthesia who had lower CSF Aβ/Tau ratios had a higher risk of postoperative delirium[41].

There are also indications that surgery, *per se*, induces changes in AD biomarkers. Patients were found to have increased levels of CSF Aβ1-42 after cardiac surgery[42]. Anckarsäter found increased concentrations of CSF t-Tau and no change in the CSF level of Aβ1-42 after knee arthroplasty[43].

1.4 Brain and Barrier

1.4.1 History and background

Evolution has gone to great lengths to protect the brain from harm. The brain is surrounded by a thick skull and immersed in protective CSF, both of which are important defenses against physical injury. The blood-brain barrier (BBB), which protects the brain from circulating toxins and pathogens, arises from the selective properties of the capillary vessels in the CNS, acting to restrict the entry of potentially harmful substances, while allowing the passage of necessary nutrients.

The concept of the BBB emerged from the late 19th Century experiments of the German physician, and later Nobel Prize winner, Paul Ehrlich. Injecting dye into the bloodstream of a mouse, he found that the dye did not stain the parenchyma of the brain. This led his student, the Berlin physician Lewandowski, to formulate the theory that the capillary wall in the brain forms a barrier to certain molecules. In the 1960s, the invention of electron microscopy allowed researchers to visualize the key structures in the morphology of the barrier, the endothelial tight junctions.

1.4.2 The neurovascular unit

The neurovascular unit (NVU) is a dynamic structure in the CNS that consists of vascular endothelial cells, pericytes, astrocyte endfeet, and neurons (Figure 1). It has a critical function in regulating CNS homeostasis by modulating cerebral blood flow, and by influencing the permeability properties of the BBB.

Blood vessels consist of endothelial cells and mural cells (mainly pericytes). The endothelial cells are largely responsible for maintaining the properties of the BBB. In the CNS, they are held together by tight junctions, creating a high-resistance barrier and limiting the paracellular flux of molecules and ions. This movement is instead mainly controlled by two types of active transport mechanisms. The first type consists of various efflux pumps, transporting lipophilic substances back to the intravascular lumen and thus preventing diffusion across the cell membrane. The second type takes care of the transport of nutrients into the CNS and the removal of waste products therefrom.

Residing on the abluminal surface of the endothelium, the pericytes contain contractile proteins that allow them to contract so as to control the diameter of the capillary, and thus the blood flow.

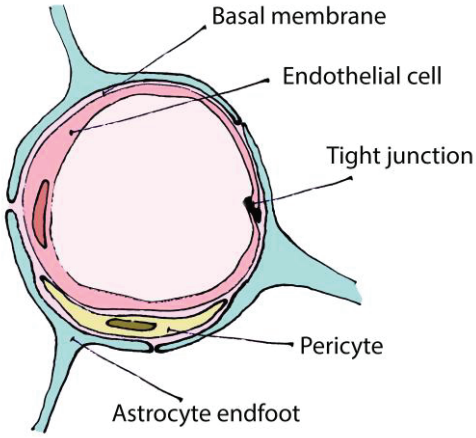

Figure 1. The neurovascular unit

The astrocytes are the conveyors of neuronal signaling to the vasculature. They have specialized processes (endfeet) that extend from the cell body to cover most of the basement membrane surface area surrounding the endothelial cells and pericytes. Connections between the astrocyte and neuron allow for the coordination of blood flow with neuronal activity. Astrocytes also secrete factors with barrier-promoting or barrier-disrupting effects depending on their interactions with neurons and endothelial cells.

1.4.3 Immune privileged organ?

The term "immune-privileged" implies that certain sites in body, e.g., the placenta / fetus complex and the testicles, have the ability to harbor antigens without eliciting an inflammatory response.

In the healthy organism, the CNS has an extremely low level of immune surveillance, the parenchyma being almost devoid of lymphocytes and neutrophils. The concept of the CNS as one of the immune-privileged organs has, however, been challenged. A growing body of evidence suggests that there is a strong linkage between systemic immune responses and the cells of the CNS.

Microglia are derived from peripheral macrophages and display a ramified morphology in their CNS resting state. They are believed to scan continuously the surrounding environment to detect extracellular changes in the environment that might be harmful to neural cells. When activated, the microglia undergo morphologic changes, shifting from their ramified phenotype to becoming enlarged and stumpy. They then proliferate, acquire phagocytic abilities, and release proinflammatory cytokines. This is normally a self-limiting process that is designed to remove cellular debris and restore homeostasis.

The endothelial cells of the NVU have an extremely low expression level of leukocyte adhesion molecules (LAMs), such as e-selectin and intercellular adhesion molecule-1 (ICAM-1) [44]. This prevents the entry of immune cells from the blood into the parenchyma under normal circumstances.

1.4.4 BBB dysfunction

It is now evident that disruption of the BBB is a major component of many neurological disorders, including stroke, dementia, traumatic brain injury, and multiple sclerosis[45]. In the evolutionary perspective, this was probably a beneficial response to, for example, trauma, a self-limiting process of healing and restoration. However, given the new spectrum of diseases in the modern era, BBB disruption may be deleterious, leading to dysregulation, altered signal homeostasis and inappropriate inflammation in the CNS, progressing to neuronal dysfunction and neurodegeneration.

During neuroinflammatory diseases, leukocyte adhesion molecule (LAM) expression on the vascular endothelial cells is increased, thereby promoting leukocyte migration across the endothelium[46]. As part of the inflammatory reaction, astrocytes release matrix metalloproteinases (MMPs), which have been shown in animal models to promote the breakdown of tight junctions and the basal membrane, thereby allowing circulating immune cells to enter the brain[47].

1.4.5 Surgery and the BBB

Animal studies have shown how peripheral surgery activates the innate immune system[48], inducing a rapid BBB disruption *via* the proinflammatory cytokine tumor necrosis factor-α (TNF-α), facilitating macrophage migration into the CNS and resulting in decline in cognitive function, a process that was prevented by disabling the action of TNF-α[32].

Reinsfelt and coworkers found that cardiac surgery triggered biochemical signs of BBB dysfunction, measured as an increase in the ratio of CSF-albumin to serum-

albumin[49]. Furthermore, cardiac surgery is known to be associated with a transient cerebral edema[50], possibly as a result of increased BBB permeability[51].

1.4.6 Assessing BBB function

The 66.5-kDa protein albumin is widely accepted as the optimal candidate marker for measurement of BBB function. This is because it is synthesized in the liver, it is not catabolized within the CNS, and it is unable to diffuse across the intact BBB. The albumin concentrations in the CSF are dependent upon active transport across the vascular endothelium, in a vesicle-mediated transcellular movement known as transcytosis. Consequently, the Gold standard for functional assessment of BBB status is to measure the CSF-albumin to serum-albumin ratio.

Different imaging techniques have been used to detect BBB damage, potentially adding information as to the location of the leakage. Computed tomography (CT) has the drawback of involving a rather high level of exposure to x-rays. Positron emission tomography (PET) requires the use of an isotope with a short half-life. Magnetic resonance imaging (MRI), which is increasingly used, uses a paramagnetic gadolinium-based contrast agent whose molecules leak from the intravascular space to the interstitial space depending on the extent of BBB damage [52].

1.5 Inflammation

A basic concept of any living organism is the constant striving to maintain homeostasis and structural integrity. The inflammatory reaction is the innate immune system's ancient and hardcoded response to a wide variety of perceived dangers. This rapidly induced first line of defense is triggered by both endogenous and exogenous factors, such as tissue damage and microorganisms, respectively.

Figure 2 shows how the inflammatory reaction can be initiated by diverse signals, including pathogen-associated molecular patterns (PAMPs), which comprise evolutionarily conserved microbial-specific components shared by most pathogens (e.g., lipopolysaccharide (LPS) and bacterial DNA), and damage-associated molecular patterns (DAMPs), which are released by damaged and dying cells. These patterns are recognized by a limited number of invariant pattern recognition receptors (PRRs), which activate microbicidal and proinflammatory responses and trigger the phylogenetically younger adaptive immune system. PRRs are expressed not only on the hematopoietic cells involved in innate immune responses (including macrophages, mast cells and neutrophils), but also on vascular and epithelial cells. The succeeding inflammatory cascade has local as well as systemic effects.

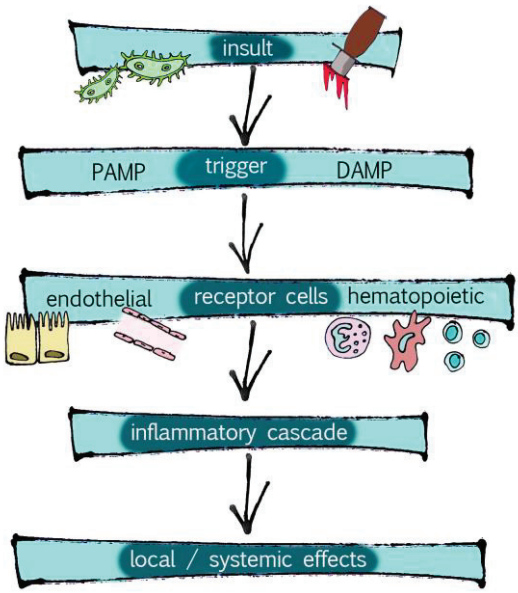

Figure 2. The inflammatory reaction

The local symptoms of inflammation were described by the Roman scholar Aulus Cornelius Celsus, in the 1st Century AD, as having the cardinal signs of *rubor* (redness), *tumor* (swelling), *calor* (heat), and *dolor* (pain).

The systemic effects include elevated body temperature, effects on the circulatory system, as well as the behavioral change known as sickness behavior, which is characterized by lethargy, depression, malaise, loss of appetite and sleepiness [53]. All of these responses are geared to reorganize the organism's priorities to cope with trauma

and disease. In severe and unresolved cases, inflammation can result in persistent organ dysfunction and, subsequently, death.

1.5.1 Inflammation and surgery

Major surgery exposes us to trauma of an extent that, set in an evolutionary perspective, is equivalent to certain death, so we cannot assume that the systemic response is appropriate in today's setting. Surgery and other aseptic traumas initiate a profound systemic inflammatory response[54], which ultimately is designed to promote healing and the restoration of homeostasis.

1.5.2 Inflammation and cardiac surgery

Cardiac surgery has some features that potentially lead to even more pronounced inflammation than is seen with general surgery. In addition to the release of DAMPs from the surgery *per se*, CPB exposes the blood to foreign materials in the extracorporeal circuit, which causes significant systemic inflammation and increases the levels of proinflammatory cytokines[55, 56]. The heparin-protamine complex formed as a consequence of anticoagulant reversal after CPB can activate the complement cascade[57]. Cardiac ischemia/reperfusion injury induces cytokine and chemokine production[58]. In a significant number of patients, this situation develops into a postoperative systemic inflammatory response syndrome with fever, organ dysfunction, and cardiovascular instability[55].

As part of the routine care in many cardiac surgery centers, potent and long-acting steroids are administered prophylactically to attenuate this systemic inflammatory response. Nevertheless, two large randomized trials on the preventive effects of steroids in cardiac surgery have shown no reduction in postoperative mortality or major morbidity compared with placebo[59, 60]. In contrast, in a 2017 study, Glumac and coworkers demonstrated a reduced risk of early (5–7 days) POCD after treatment with a lower dose of dexamethasone administered 10 hours before cardiac surgery[61].

1.5.3 Neuroinflammation

Blood-borne inflammatory mediators invoke degradation of the BBB, migration of monocyte-derived macrophages into the brain parenchyma, and a

neuroinflammatory response[32]. Increased levels of inflammatory cytokines are detected in the CSF after general surgery[62], as well as after cardiac surgery[49].

In the first imaging study of postoperative neuroinflammation, Forsberg and colleagues used positron emission tomography (PET) and a radioligand that was selective for the translocator protein expressed by microglia[63]. A suppressive response on Days 3–4 was followed by microglial activation at 3 months in the subset of patients with cognitive decline.

1.6 Biochemical markers

Markers of inflammation and CNS affection are measured in the blood and in the CSF. CSF, which is the clear liquid surrounding the brain and spinal cord, is secreted by the choroid plexuses located in the cerebral ventricles. The CSF has a high turnover rate with a volume of 80–150 ml and a production level of 500 ml/day. Some proteins with high expression within the CNS are also detectable in the blood, albeit at very low concentrations.

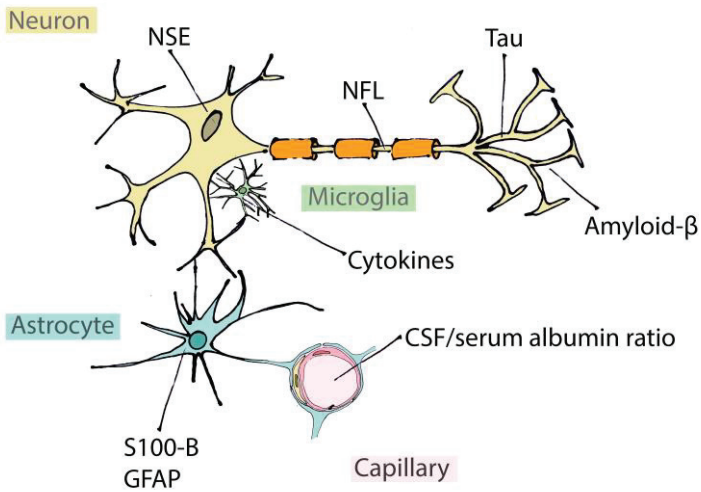

Figure 3. Biomarkers of BBB function, neuronal inflammation and injury

1.6.1 Inflammatory biomarkers

The inflammatory cascade involves numerous substances (cytokines) with diverse, more or less well-known roles. Maintaining the balance between pro-and anti-inflammatory factors is a delicate task and many mediators seem to have counteracting functions in different phases of the inflammatory process.

Interleukin-6 (IL-6), IL-8, IL-1β and TNF-α are among the most extensively studied cytokines in the surgery-induced inflammation setting. Substantial increases in the levels of pro- and anti-inflammatory biomarkers are detected in the serum and CSF after knee and hip replacement surgery[62], as well as after cardiac surgery [49].

1.6.2 S-100B

S-100B is a calcium-binding protein that is present mainly in the cytoplasm of mature perivascular astrocytes with a serum half-life of 20–25 minutes. It is, however, also expressed in non-neuronal cells, such as hepatocytes, myocytes, and melanocytes, and in adipose tissue, which means that extracranial sources can contribute to elevated serum levels of S-100B. As a marker of brain injury, it has shown correlations to both CT findings and unfavorable functional outcomes after traumatic brain injury[64]. S-100B is present at increased levels after cardiac surgery, as a sign of glial cell injury and BBB damage[49].

1.6.3 Neuron-specific enolase

NSE is an enzyme that is involved in glycolysis, and is, despite its name, present not only in neurons but also in erythrocytes and endocrine cells. Its high sensitivity to hemolysis severely limits its usefulness as a brain injury biomarker, particularly in cardiac surgery. However, the CSF level of NSE correlates with mortality after severe traumatic brain injury[65]. The half-life of NSE in serum is estimated to be 30 hours.

1.6.4 Tau

Tau is a family of neuronal proteins, having 6 isoforms that are involved in the assembly and maintenance of the microtubule structures in neuronal cells. The Tau protein is abundant in the thin, nonmyelinated axons of cortical interneurons. Aggregations of insoluble phosphorylated Tau (p-Tau) in neurofibrillary tangles (NFTs), are a diagnostic marker of AD.

Total-Tau (t-Tau) in CSF is correlated with outcome in patients with traumatic brain injury[66], and CSF t-Tau is also elevated in AD. Plasma t-Tau and CSF t-Tau levels have been shown to have a poor correlation to each other[67].

1.6.5 Neurofilament light chain

Neurofilaments are, like microtubules, part of the neuronal cytoskeleton. The neurofilament light chain (NFL) subunit, together with its medium and heavy counterparts, determine the axonal caliber and, thus, the axonal velocity. NFL is expressed exclusively by central and peripheral neurons and is most abundant in the large-caliber myelinated axons that project into deeper brain layers and the spinal cord. There is a strong correlation between the plasma and CSF levels of NFL[68].

NFL is known to have a serum half-life of several weeks[69]. Following ischemic stroke, the levels of NFL in the CSF and serum increase continuously during the first 3 weeks[70]. In a study of patients after neurosurgical trauma, CSF and serum NFL peaked after 1 month[71].

1.6.6 Glial fibrillary acidic protein

GFAP is a CNS-specific cytoskeletal protein that is almost exclusively expressed in astrocytes. It is not yet as well studied as S-100B but the levels of GFAP have been correlated to the severity of traumatic brain injury[72].

1.6.7 Amyloid-β (Aβ)

Aβ1-42, which is the 42-amino acid isoform of β-amyloid, is the major component of senile plaques, which are characteristic of AD pathology. Aβ1-42 is a cleavage product of amyloid precursor protein (APP) with no known physiologic function. While APP is expressed and metabolized in many cell types, it is concentrated in the neuronal synapses, where Aβ1-42 secretion is at the highest level.

Patients with AD have decreased levels of CSF Aβ1-42, and these levels are inversely correlated to the number of amyloid plaques. Plasma Aβ1-42 is emerging as a possible AD screening tool, although extracerebral sources may make interpretation difficult, as a significant source of systemic Aβ seems to be activated platelets [73].

2. Aims

I. To investigate whether orthopedic surgery or cardiac surgery induces permeability changes in the BBB

II. To investigate the systemic and neuroinflammatory responses to cardiac and orthopedic surgeries

III. To define the association between the neuroinflammatory response and long-term postoperative decline in patients after orthopedic surgery

IV. To determine whether orthopedic surgery causes neural injury or brain amyloidosis

V. To examine the biochemical evidence for neuronal damage or brain amyloidosis as a mechanism for cognitive decline after orthopedic surgery

VI. To assess the potentials of preoperative biomarkers of neuroinflammation, brain injury and amyloidosis to predict unfavorable neurocognitive outcomes from orthopedic surgery

VII. To test whether a perioperative single dose of methylprednisolone can attenuate the proposed postoperative BBB dysfunction and prevent neuroinflammation after cardiac surgery.

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3. Patients and methods

Recruiting patients for clinical studies can be a challenging task, especially when the studies involve invasive procedures, such as CSF collection. Thus, a common denominator in these studies is that many patients were called, but few were chosen (or rather choose to participate).

3.1 Papers I and II

Papers I and **II** are both concerned with the NEUPORT study and will thus be described together.

3.1.1 Study design

The NEUPORT study was a prospective, observational, two-center investigation. The study protocol was reviewed and approved by the Regional Ethics Committee in Stockholm, Sweden (Dnr. 2013/2297-31/4, 2014/834-32), and the study was registered at www.clinicaltrials.gov (Id: NCT02759965).

3.1.2 Inclusion, exclusion criteria

Patients who were scheduled for total hip or knee arthroplasty at either Karolinska University Hospital, Stockholm or Sahlgrenska University Hospital, Mölndal were screened for eligibility. After providing signed informed and written consent, 34 patients were eventually included in the study. As the focus of the study was neuropsychological outcomes and neuroinflammation, there was an extensive list of exclusion criteria, including neurologic, psychiatric or neurovascular disease, treatment with anti-inflammatory drugs, severe organ failure, coagulopathy, alcohol or drug abuse, poorly controlled diabetes mellitus, or autoimmune disease. Patients were preoperatively screened using the Mini Mental State Exam (MMSE) and patients with cognitive impairment were excluded. Of the 34 patients included in the study, 7 were excluded. Twenty-seven patients were analyzed. Six of the patients had cognitive deficits after 3 months and constitute the *Poor neurocognitive outcome* group, while the

remaining 21 patients made up the *Good neurocognitive outcome* group. Twenty-four patients with complete datasets were included in the PCA analysis (see Section 3.5.1)

3.1.3 Experimental protocol

A schematic of the experimental protocol is presented in Figure 4.

Cognitive performance was assessed on three occasions (at inclusion in the study, at discharge from the hospital, and at 3 months postoperatively) using the International Study of Postoperative Cognitive Dysfunction (ISPOCD) test battery (see Section 3.4)

Prior to the operation, a lumbar spinal catheter was inserted, left in place for 48 hours and used for collecting CSF samples at the specified time-points. Spinal anesthesia was administered using the spinal catheter and light sedation was achieved with propofol infusion. Six patients received subcutaneous ketorolac intraoperatively as part of the postoperative pain regimen, otherwise anti-inflammatory and psychotropic drugs were avoided.

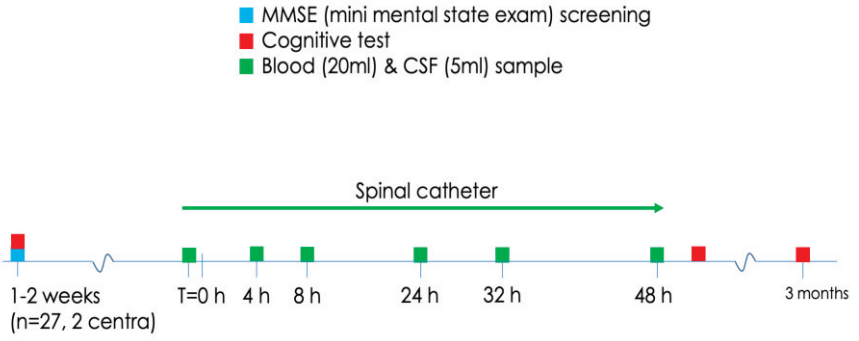

Figure 4 Protocol for studies described in Papers I and II

3.2 Paper III

3.2.1 Study design

We conducted a prospective, randomized, double-blinded, double-armed study. A randomized block design was used based on gender and type of surgery.

The study was approved by The Gothenburg Regional Ethics Committee and the Swedish Medical Product Agency, and was registered at www.clinicaltrials.gov (id: NCT01755338)

3.2.2 Inclusion, exclusion, randomization, and blinding criteria

Patients were assessed on admission to the hospital and were given information and asked for consent (oral and written) by one of the investigators. Eligible patients were those who were scheduled for elective open aortic valve replacement surgery with a bioprosthesis due to aortic stenosis with or without concurrent coronary artery bypass grafting and normal preoperative left ventricular function (>50%). Patients with a history of stroke were excluded. To minimize the risk for complications after lumbar puncture, no patients with recent treatment with thrombolytic or potent antiplatelet drugs were included, and their preoperative and postoperative coagulation tests had to be normal.

After inclusion, the 30 patients were randomized to two well-matched groups (Table 1 in **Paper III**), equal in size, using selection of closed envelopes. Preparation of the drugs was done by a nurse who was otherwise neither involved in the study nor in the care of the patients.

3.2.3 Experimental protocol

A schematic of the experimental protocol is presented in Figure 5.

On two occasions, the day before surgery and approximately 24 hours after surgery, a lumbar puncture was performed and the obtained CSF sample, together with a blood sample, was sent to the neurochemistry laboratory, where it was centrifuged, aliquoted, and stored at −80°C for later analysis.

After induction, the patients received the study drug (either methylprednisolone 15 mg/kg bodyweight or placebo (an equal volume of 0.9% Sodium Chloride).

Anesthesia, monitoring and cardiopulmonary bypass were in both groups performed as per the routine praxis of our unit.

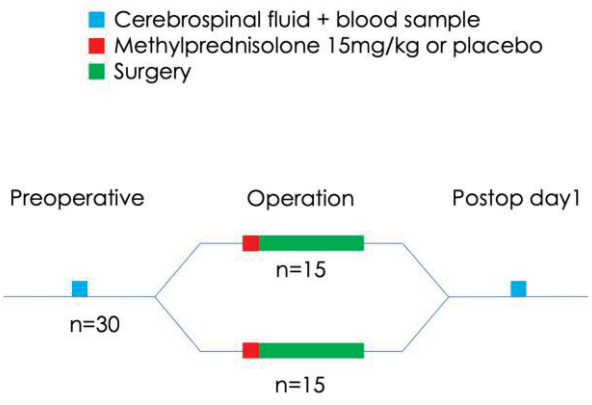

Figure 5 Study protocol for Paper III

3.3 Serum and CSF biomarker analyses

In **Paper I**, the samples (CSF and serum) were analyzed using a high-throughput, multiplex immunoassay analysis (Proseek Multiplex, Proximity Extension Assay technology, Inflammation 1 panel; Olink, Uppsala, Sweden) for the 92 established and exploratory inflammatory biomarkers using DNA-labelled antibody probe pairs. The antibodies bind to proteins in the sample and, in the next step, a polymerase chain reaction (PCR) reporter sequence is formed through a proximity-dependent DNA polymerization event. The sequence is amplified, and subsequently detected and quantified using Real-Time PCR. The analyzed proteins are listed in Appendix table 1. The values for the various inflammatory variables are presented as arbitrary units in Log_2 scale and can thus be used only for evaluating relative changes in the levels of the same protein.

CSF and serum S-100B were assayed using an Electrochemiluminescence (ECL) immunoassay using the Modular system and the S-100B reagent kit (Roche Diagnostics, Basel, Switzerland).

Glial fibrillary acidic protein (GFAP) was measured using an ELISA method [74].

CSF- and serum NSE were measured using an immunofluorescent assay with the time-resolved amplified cryptate emission (TRACE) technology (Kryptor-NSE; BRAHMS GmbH, Hennigsdorf, Germany).

The CSF total-Tau (t-Tau) concentration was determined using a sandwich ELISA (INNOTEST hTAU Ag; Fujirebio Europe NV, Gent, Belgium), which measures all tau isoforms irrespective of phosphorylation status.

The CSF-NFL concentration was determined using an enzymatic two-site immunoassay (UmanDiagnostics NF-light® assay; UmanDiagnostics AB, Umeå, Sweden).

CSF-Aβ1-42 were determined using a sandwich ELISA (INNOTEST® β-amyloid (1-42), Fujirebio, Belgium).

The plasma concentrations of t-Tau and Aβ1-42 and the serum concentration of NFL were measured using a single-molecule array technology, as described by the manufacturer (Quanterix, Billerica, MA), and in **Paper II**, the blood to CSF ratios of these proteins were subsequently calculated.

Albumin levels in the CSF (mg/L) and serum (g/L) were measured by immunonephelometry on the IMMAGE immunochemistry system (Beckman Coulter Inc., Fullerton, CA, USA), and the CSF-albumin/serum-albumin ratio was subsequently calculated.

The serum and CSF levels of TNF-α, IL-6 and IL-8 in **Paper III** were determined using the Human Proinflammatory II 4-Plex Assay, Ultra-Sensitive Kit, with electrochemiluminescent detection (Meso Scale Discovery®; Meso Scale Daiagnostics Inc. Rockville, MD, USA.

3.4 Cognitive tests

To assess cognitive capacity, we used the ISPOCD test battery, in which the following four tests (Test 1 is repeated in the end) are used to measure different aspects of cognitive performance[7]: 1) The visual verbal learning test, based on Rey's auditive recall of words[75], which tests memory capacity. Fifteen words are presented at a fixed rate on a computer monitor. The patients are asked to recall as many words as possible. 2) The concept shifting test, which is based on the trail making test of Halstead and Reitan[76]. This tests the ease of shifting between two similar tasks. 3) The Stroop Color-Word Interference Test[77], which tests for speed and attention. 4) The letter-digit coding test, which is based on the symbol-digit substitution task from the Wechsler adult intelligence scale[78]. It tests the subjects' speed of visual information processing. 5) Finally, the patients were tested for delayed recall of the 15 words from the visual verbal learning test (Test 1).

The tests were carried out in a quiet room with only the patient and investigator present. Investigators were trained by the group responsible for development of the test battery.

3.5 Statistical analysis

3.5.1 Paper I

Statistical analysis was performed using the SPSS for Macintosh and SAS version 9.4 software.

For the Power, analysis we hypothesized that there would be a significant association between the postoperative IL-6 concentration in the CSF and a change in cognitive function (combined z-score) at the first postoperative test. A correlation coefficient of 0.5 for the association between CSF IL-6 concentration and the z-score would require 25 patients to obtain a power of 80%.

Group differences in the pre- and peri-operative characteristics were tested using the Mann–Whitney *U*-test and Fisher's exact test. A 2-way analysis of variance (ANOVA) for repeated measurements was used to assess differences between the groups with respect to quality of sleep and the severity of postoperative pain (VAS).

Data are presented as either mean ± standard error of the mean (SEM), median with interquartile range (IQR) or median (Q1-Q3) when appropriate. Changes over time were tested by a repeated measurements ANOVA, and paired *t*-tests were used to assess significant changes from the preoperative levels at each postoperative time-point.

A principal component analysis (PCA) was performed on the preoperative CSF and blood biomarkers. Only those markers for which more than 75% of the values were above the level of detection (52 of 92) were included in the PCA. A PCA can be regarded as a tool for compressing a large, high-dimensional dataset while retaining most of the variation. Principal components (PCs) are identified, along which the variation in the data is maximal. The impacts of the different markers on the PC are reflected in the weights. The three first PCs were selected as primary outcome variables and used to create postoperative outcome variables by applying their weights to the Z-transformed markers at each postoperative time-point for each variable. The results were then subjected to a mixed model analysis to detect differences between the patients with or without long-term cognitive decline. The analyses of individual CSF and blood biomarkers were carried out with a similar model. To compare proportions with positive slopes in the PC2 analysis with respect to cognitive outcome,

we used Fisher's exact test. When testing the preoperative biomarkers for between-group differences, a Bonferroni-Holm correction was applied.

3.5.2 Paper II

Statistical analysis was performed using the SPSS for Macintosh software.

For the statistical analyses of the pre-, peri-, and post-operative patient characteristics, see Section 3.5.1 in **Paper I**. Data are presented as either mean ± standard deviation (SD) or median with interquartile range (IQR) when appropriate.

Postoperative longitudinal changes in the levels of the biomarkers of neuronal injury independent of group were analyzed using ANOVA for repeated measurements. A group-time interaction analysis of covariance (ANCOVA) of the various neuronal injury markers was used to compare groups with poor or good cognitive outcomes at 3 months post-surgery, using the baseline measurements as a covariate to adjust for baseline differences between the groups.

To study the correlation between CSF and blood levels of T-tau and NFL, Pearson correlation coefficients were calculated.

A post-hoc Power analysis revealed that the power to detect a 40%–50% difference in peak postoperative CSF t-Tau between the groups was 0.69–0.87 at a SD of 225 pg/ml. The power to detect a 40%–50% difference in peak CSF Aβ1-42 was 0.64–0.82 at a SD of 275 pg/ml.

3.5.3 Paper III

Statistical analysis was performed using the SPSS for Macintosh software.

A Power analysis was performed based on our previous study[49]. To detect a 50% difference in CSF IL-8 (primary endpoint) between the groups, with a power of 0.80 and α-value of 0.05 and a SD of 60 ng/L, 13 patients were needed in each group. Continuous variables were examined for normal distribution using the Shapiro-Wilk test and within-group differences were compared using a paired Student's *t*-test or Wilcoxon signed-rank test when appropriate. The comparison of the placebo and intervention groups was tested using an unpaired *t*-test test or Mann-Whitney *U*-test. All data are presented as either mean ± standard deviation (SD), median with interquartile range (IQR) or median (Q1-Q3) when appropriate. A probability level (p-value) of less than 0.05 was considered to indicate statistical significance.

4. Results

4.1 Patient populations

Paper I and II

An overview of the clinical trial flowchart for **Papers I** and **II** is presented in Figure 6. Overall, 156 patients were assessed for eligibility and 34 patients were included. Of these, 7 patients were not analyzed for reasons described in Figure 6, resulting in 27 patients in the final analysis.

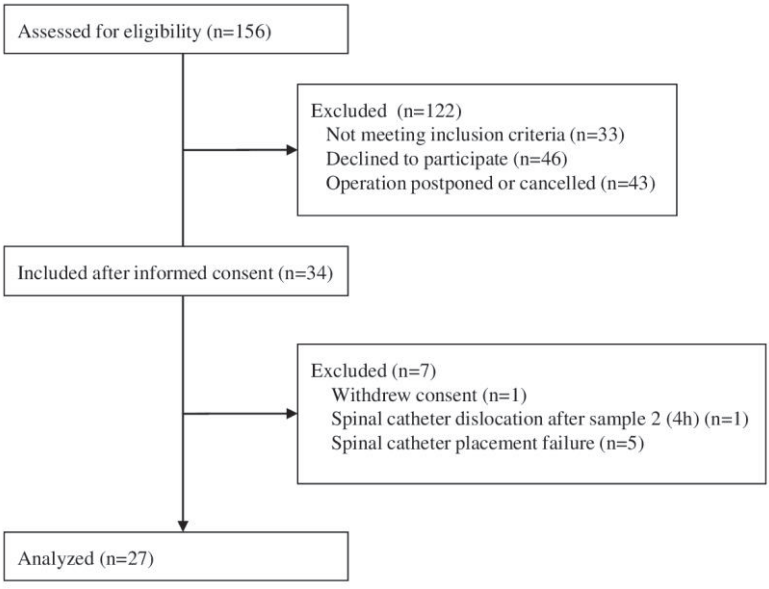

Figure 6. CONSORT diagram showing patient inclusion (Papers I and II). Source: Danielson, M. et al., Ann Neurol, 2020, Creative Commons BY 4.0 License

The median age of the participants was 71 (10) years and the male/female ratio was 9/18. According to the physical status classification system of the American Society of Anesthesiologists (ASA), the patients were distributed as ASA I (healthy

patient, 26%), ASA II (mild systemic disease, 70%) and ASA III (severe systemic disease, 4%). Patient demographics and comorbidities are detailed in Table 1.

Characteristics	Good neurocognitive outcome (n=21)	Poor neurocognitive outcome (n=6)	p-value
Preoperative			
Age, years	71 (65-76)	68 (65-71)	0.32
Sex, male, n (%)	8 (38)	1 (17)	0.63
Weight, kg	80 (73.5-89)	79 (75-88.5)	0.93
Height, cm	171 (167-179)	166 (163-175)	0.24
Body Mass Index, kg/m2	26.7 (24.6-29.4)	27.9 (25.7-31.5)	0.44
Comorbidity			
Hypertension, n	11	4	0.66
Diabetes mellitus, type 1, n	2	0	1
Diabetes mellitus, type 2, n	0	1	0.22
Myocardial infarction, n	1	0	1
ASA classification I/II/III/IV, n	7/13/1/0	0/6/0/0	0.197
Blood hemoglobin, g/l	138 (128-147)	143 (134-157)	0.41
Serum creatinine, µmol/l	71 (62-89)	68 (47-96)	0.63
Preoperative WBC count	6.3 (5.4-7.9)	7.7 (6.5-8.7)	0.22
Intraoperative			
Medication			
Propofol, mg	170 (80-291)	168 (75-288)	1.0
Fentanyl, n	4	0	0.55
Alfentanil, n	2	0	1.0
Vasopressor, n	12	5	0.36
Intravenous fluids, ml	1200 (950-1350)	925 (750-1560)	0.44
Duration of procedure, minutes	89 (72-102)	86 (72-101)	0.93
Procedure			
Hip replacement, n	14	5	0.63
Knee replacement, n	7	1	0.63
Bleeding, ml	300 (150-450)	225 (150-550)	1.0
Postoperative (24 hours)			
PACU length of stay, minutes	165 (98-225)	171 (128-579)	0.47
Intravenous fluids, ml	1000 (750-1850)	1575 (260-2500)	0.48
Medication			
Gabapentin, n	6	2	1
Oral opioid, n	20	6	1
Intravenous opioid, n	13	3	0.66
Post spinal puncture headache, n	0	1	0.22
Blood patch, n	0	1	0.22

Table 1. Demographic and intraoperative characteristics (Papers I and II). Values are median (Q1-Q3). Group differences tested by Mann–Whitney U test and Fisher exact test.

The Good and Poor neurocognitive outcome groups did not differ in terms of demographic variables, overall comorbidity burden, preoperative laboratory results or ASA physical status level. Furthermore, no differences were detected with respect to the intraoperative or postoperative use of sedatives, vasoactive drugs or intravenous fluids. Bleeding, duration of operation and stay in the post-anesthesia care unit,

as well as assessment of pain and sleep quality (VAS score) did not differ between the two groups. One patient developed post-spinal headache, which was successfully treated with a blood patch. Otherwise, the post-operative period was uneventful, with none of the patients developing a postoperative delirium.

Paper III

The clinical trial profile of **Paper III** is presented in Figure 7

Sixty-six patients undergoing cardiac surgery were assessed for eligibility. Six patients did not meet the inclusion criteria, 23 patients declined to participate and in 7 patients surgery was cancelled or postponed. Finally, 30 patients were randomized to receive either placebo (n=15) or methylprednisolone (n=15).

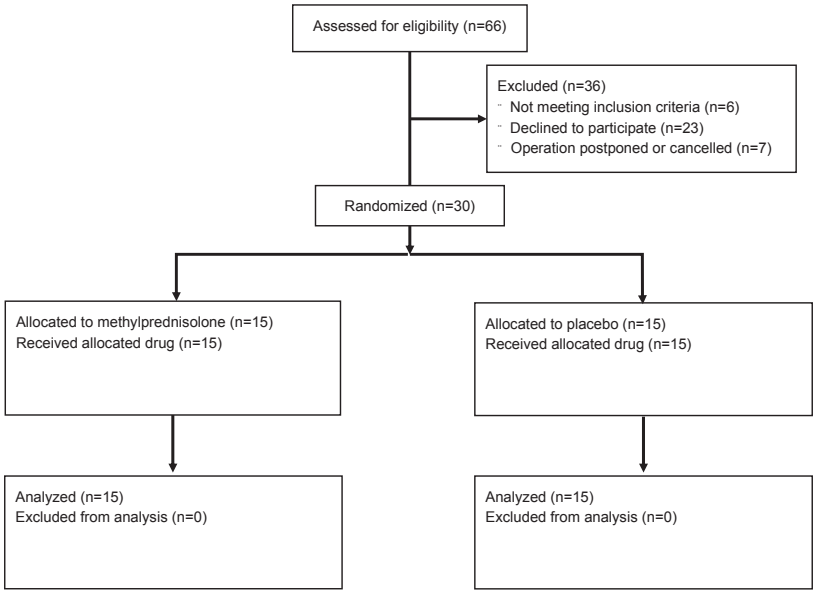

Figure 7. CONSORT diagram showing patient inclusion (Paper III). Source: Danielson, M. et al., J Neuroinflammation, 2018, Creative Commons BY 4.0 License

The patients' demographics and comorbidities are detailed in Table 2.

There was no difference between the groups with respect to age, gender, body weight, comorbidity burden, preoperative cardiac status, EuroSCORE II or type of surgery performed. There were no complications related to the preoperative or postoperative lumbar punctures. The groups were similar with respect to duration of procedure, extracorporeal circulation and aortic cross-clamp. While the perioperative and postoperative blood glucose levels did not differ between the groups, there was a statistically significant increase in insulin requirement in the methylprednisolone-treated group.

Characteristics	Placebo (n=15)	Methylprednisolone (n=15)	p-value
Demographics			
Age, years	68.9±11	70.7±7.0	
Male sex, n	13	10	
Body weight, kg	81.1±9.0	82.5±15.7	
Height, cm	173±7.0	176±7.9	
Comorbidity			
Hypertension, n	9	9	
Previous heart surgery, n	0	0	
Atrial fibrillation/flutter, n	1	0	
Serum creatinine, µmol/l	89.7±15	88.3±20	
Cardiac status			
LVEF, %	60±4.2	61±5.8	
NYHA			
I (n)	7	4	
II (n)	4	9	
III (n)	4	2	
IV (n)	0	0	
EuroSCORE II, %	1.8±1.4	1.9±1.5	
Type of surgery			
SAVR, n	10	11	
CABG + SAVR, n	5	4	
Intraoperative data			
Duration of procedure, minutes	189±43	204±58	0.43
Duration of CPB, minutes	99.4±25	115±31	0.14
Duration of aortic cross-clamping, minutes	81.5±25	91.7±29	0.28
Intra-operative dose of phenylephrine, mg	1.02±1.1	1.36±1.0	0.39
Intra-operative dose of norepinephrine, mg	0.58±1.3	0.56±0.55	0.95
Plasma glucose levels			0.096
Start of procedure, mmol/l	6.34±1.5	5.25±0.47	
During CPB, mean, mmol/l	6.52±0.94	7.25±1.0	
After CPB, mmol/l	7.15±0.97	8.50±1.7	
Arrival ICU, mmol/l	6.20±0.5	7.20±1.6	
Day 1 ICU, mmol/l	6.27±1.2	6.44±1.2	
Insulin demand U/hour, intraoperatively + ICU	1.14±0.74	2.46±1.27	0.009**
Postoperative data			
Serum creatinine, µmol/l	83.9±18	94.9±21	0.14
Vasopressor treatment, n	6	9	0.47
Inotropic treatment, n	0	0	1.0
Reoperation for bleeding, n	0	0	1.0
ICU readmission, n	0	1	1.0
Atrial fibrillation/flutter, n	5	10	0.14
Neurologic complication, n	2	1	1.0
Pneumonia, n	0	1	1.0
Wound infection, n	1	1	1.0
Other infection, n	0	2	0.48
Length of stay in ICU, hours	24.8±14	22.7±12	0.65

*Table 2. Demographics, intraoperative and postoperative characteristics of the patients (Paper III). Values shown are mean ± SD. **p < 0.01*

There was no difference in postoperative serum creatinine, vasopressor or inotropic treatment, incidence of reoperation for bleeding, risk of ICU readmission, incidence of atrial fibrillation/flutter, neurologic complications, incidence of infections, or length of ICU/hospital stay.

4.2 Neurocognitive outcome (Papers I and II)

The results for neurocognitive outcome after major orthopedic surgery are shown in Figure 8. When discharged from the hospital (3–5 days post-surgery), 78% (21 patients) had a composite z-score of ≥1.0. At the evaluation 3 months post-surgery, 22% (6 patients) showed a composite z-score ≥1.0 (Poor neurocognitive outcome group, n=6). The remaining 21 patients (78%) had a composite z-score of <1.0 (Good neurocognitive outcome group, n=21).

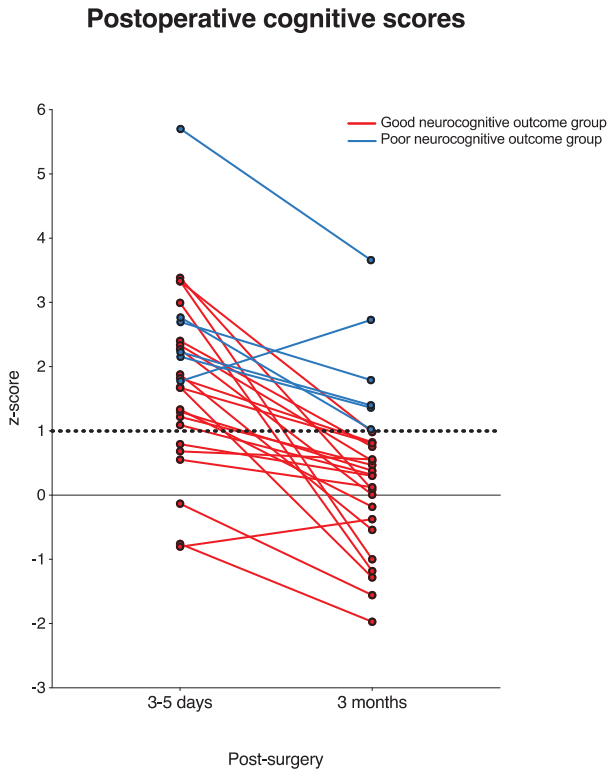

Figure 8. Neurocognitive outcomes for patients after major orthopedic surgery. Patients with a z-score of ≥1.0 at 3 months (n=6) constitute the Poor neurocognitive outcome group. Source: Danielson, M. et al., Ann Neurol, 2020, Creative Commons BY 4.0 License

4.3 Blood-brain barrier (Papers I and III)

In **Paper I**, for the patients undergoing major orthopedic surgery, there was a significant increase in the CSF-albumin to serum-albumin ratio at 4 and 8 hours postoperatively (p=0.013) (Figure 9). There was no association between these changes and neurocognitive outcome at 3 months postoperatively.

In **Paper III**, the increase in the CSF-albumin to serum-albumin ratio was more pronounced in the cardiac surgery patients than in the patients undergoing orthopedic surgery. The CSF-albumin to serum-albumin ratio increased by 20%–25%, 24 hours after surgery in both the control and methylprednisolone group, with no significant difference between the groups (Figure 9).

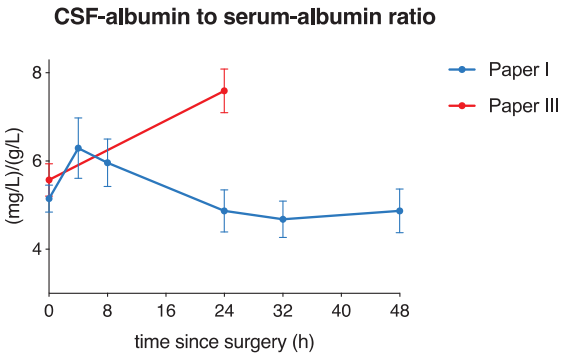

Figure 9. Ratios of CSF-albumin to serum albumin in patients pre- to post-surgery. The time-point 0 is the preoperative value. Data are presented as mean ± SD. Source: pooled data from Papers I and III.

4.4 Astrocyte injury markers (Papers I and III)

Two biomarkers of astrocyte injury, S-100B and GFAP, were studied in **Papers I** and **III**. In **Paper I**, the serum S-100B (p<0.001), CSF S-100B (p=0.028) and GFAP (p=0.005) levels all showed significant changes over time, with transient increases at 4 and 8 hours after surgery (Figure 10). These changes were not associated with neurocognitive outcome.

In **Paper III**, the CSF levels of S-100B and GFAP were not significantly increased 24 hours after surgery in the placebo group. In the methylprednisolone group, CSF S-100B (but not GFAP) increased marginally 24 hours after surgery. The serum level of S-100B increased in both groups, with no significant difference between the groups.

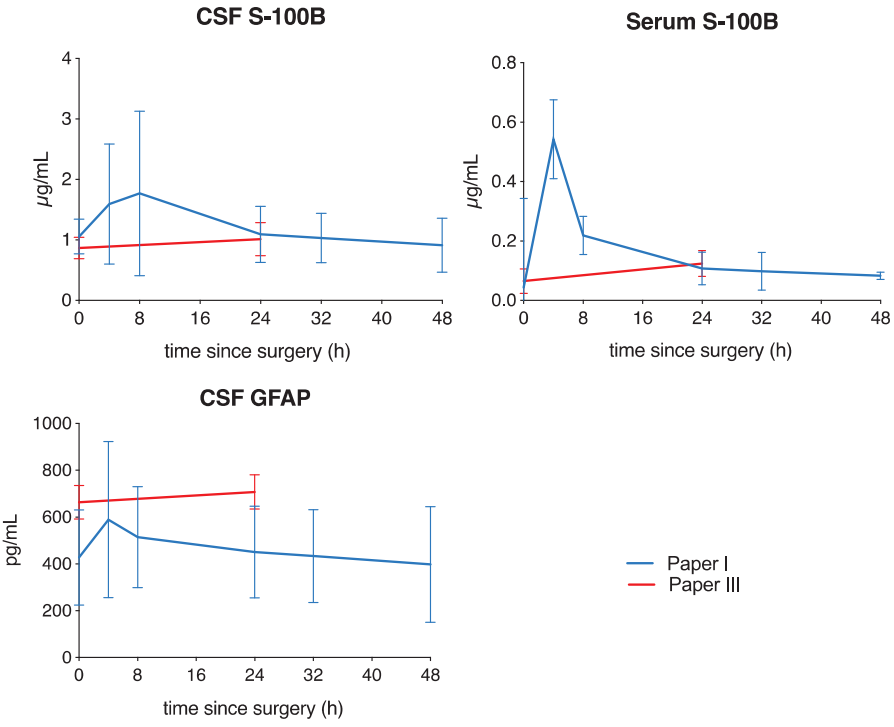

Figure 10. Concentration of astrocyte injury markers in patients pre- to post-surgery. The time-point 0 is the preoperative value. Data are presented as mean ± SD. Source: pooled data from Papers I and III

4.5 Neuronal injury markers (Papers II and III)

The CSF to blood ratios were positive for t-Tau and NFL in **Paper II** (blood concentrations were not measured in **Paper III**) while the corresponding ratio for NSE was the opposite (higher in serum), in both **Papers II** and **III**.

During the 48-hour postoperative period in **Paper II**, the level of CSF T-tau increased progressively to 33% above the preoperative level ($p<0.001$) (Figure 11).

However, the plasma levels of t-Tau showed a transient increase, peaking at 4 hours post-surgery and returning to baseline at 48 hours. There was no difference between the Good and Poor neurocognitive outcome groups in the temporal changes in the levels of either CSF t-Tau (p=0.310) or plasma t-Tau (p=0.889). The plasma T-Tau to CSF T-tau ratio was higher in the poor neurocognitive outcome group (p=0.028) (Figure 5, **Paper II**). There was no correlation between plasma and CSF T-tau (r=0.015, p=0.85, Supplemental Figure 3, **Paper II**).

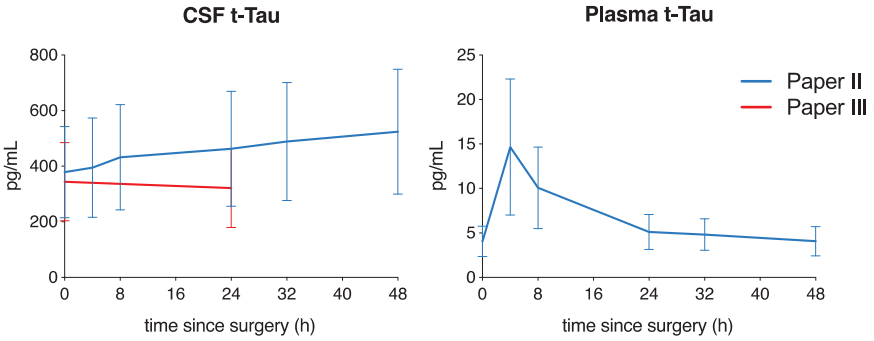

Figure 11. Concentration of t-Tau in the CSF and plasma of patients pre- to post-surgery. The time-point 0 is the preoperative value. Data are presented as mean ± SD. Source: pooled data from Papers II and III

In **Paper III**, there was a minor, albeit significant (p<0.05), increase in CSF t-Tau 24 hours after cardiac surgery in the control group. In the overall material (Figure 11), as well as in the methylprednisolone group, there was a non-significant decrease in CSF t-Tau.

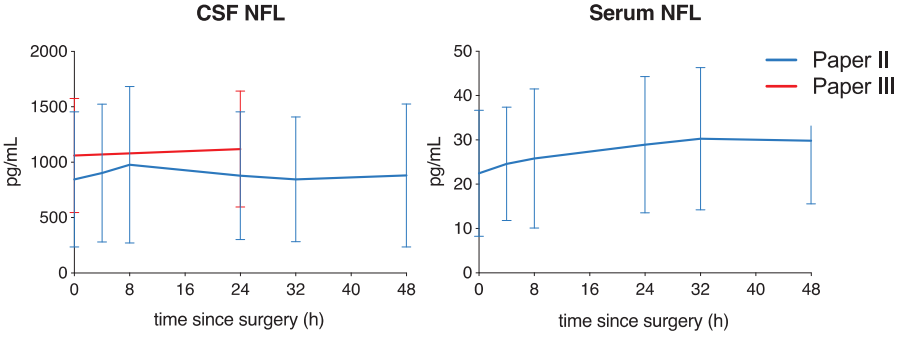

Figure 12. Concentration of NFL in the CSF and serum of patients pre- to post-surgery. The time-point 0 is the preoperative value. Data are presented as mean ± SD. Source: pooled data from Papers II and III

In the 48-hour sampling period after orthopedic surgery in **Paper III**, there was no significant change in CSF-NFL (p=0.188) (Figure 12).

The serum–NFL level showed a postoperative gradual increase up to 35% of the preoperative level at the end of the sampling period (p=0.001) (Figure 12).
There was no difference between the Good and Poor neurocognitive outcome groups with respect to changes in the levels of CSF-NFL (p=0.437), serum-NFL (p=0.112) or in the serum NFL to CSF NFL ratio (p=0.172) (Figure 5, **Paper II**). There was poor but significant correlation between serum and CSF NFL (r=0.26, p<0.001, Supplemental Figure 4, **Paper II**).

In **Paper III**, the levels of CSF-NFL were not changed in either the placebo group or the methylprednisolone group after cardiac surgery.

The CSF and serum levels of NSE both increased over the first 8–32 hours after orthopedic surgery (p=0.002 and p=0.009, respectively) (Figure 13), and thereafter declined towards the baseline level. There was no difference in NSE levels between the Good and Poor neurocognitive outcome groups in CSF (p=0.124) or serum (p=0.0612)

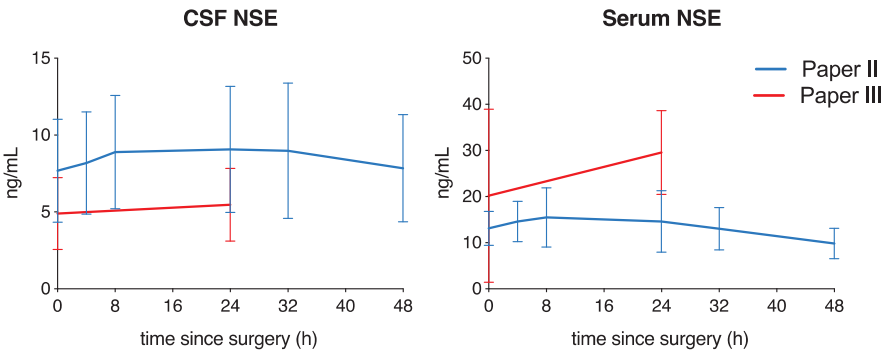

Figure 13. Concentration of NSE in the CSF and serum of patients pre- to post-surgery. The time-point 0 is the preoperative value. Data are presented as mean ± SD. Source: pooled data from Papers II and III

As shown in **Paper III**, while there was no significant change in the CSF levels of NSE, the serum levels of NSE increased in both the methylprednisolone-treated and placebo groups, with no significant between-group differences (p=0.36).

4.6 Markers of brain amyloidosis (Paper II)

In **Paper II**, there were significant changes in the plasma ($p<0.001$) and CSF ($p=0.019$) levels of Aβ1-42 over time after orthopedic surgery, with maximal levels observed at 48 hours (Figure 14). There was, however, no difference between the patients in the Good and Poor postoperative neurocognitive outcome groups with respect to the CSF ($p=0.057$) or plasma ($p=0.172$) levels of Aβ1-42. Neither was there any significant difference between the groups in terms of the preoperative levels of Aβ1-42 (616±321 and 496±217 pg/ml, respectively, for the patients in the Good and Poor postoperative neurocognitive outcome groups ($p=0.40$). The plasma Aβ1-42 to CSF Aβ1-42 ratio was higher in the poor neurocognitive outcome group ($p=0.038$) (Figure 5, **Paper II**).

An Aβ1-42 level <550 pg/ml in the CSF has been used as a diagnostic cutoff value for AD. However, this cutoff showed no prognostic value for postoperative cognitive decline, as 4/16 patients (25%) with Aβ1-42 levels <550 pg/ml and 2/11 patients (18%) with Aβ1-42 levels >550 pg/ml had poor neurocognitive outcomes ($p=1.0$). Neither was there any significant difference in preoperative T-tau levels in CSF comparing the Good (411±163 pg/ml) and Poor (253±64 pg/ml) neurocognitive outcome groups ($p=0.03$).

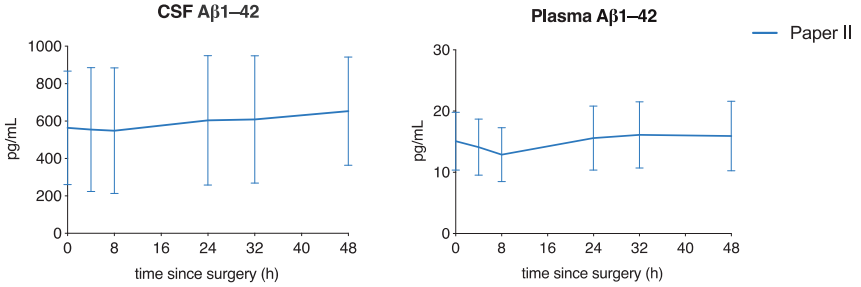

Figure 14. Concentration of Aβ1-42 in the CSF and plasma of patients pre- to post-surgery. The time-point 0 is the preoperative value. Data are presented as mean ± SD. Source: pooled data from Paper II

4.7 Markers of neuroinflammation

4.7.1 Neuroinflammation and cognitive dysfunction in orthopedic surgery (Paper I)

In **Paper I**, 92 established and experimental inflammatory markers were analyzed in CSF and serum samples at six time-points: before surgery and at 4, 8, 24, 32 and 48 hours after surgery. A PCA was performed on the pre-operative CSF and serum data, and outcome variables were constructed from the three first principal components (PC1, PC2 and PC3). The two first construct outcome variables for CSF, PC1 and PC2, were significantly associated with poor cognitive outcome after 3 months, with increases over time in PC1 ($p=0.02$) and PC2 ($p<0.001$) (Figure 15).

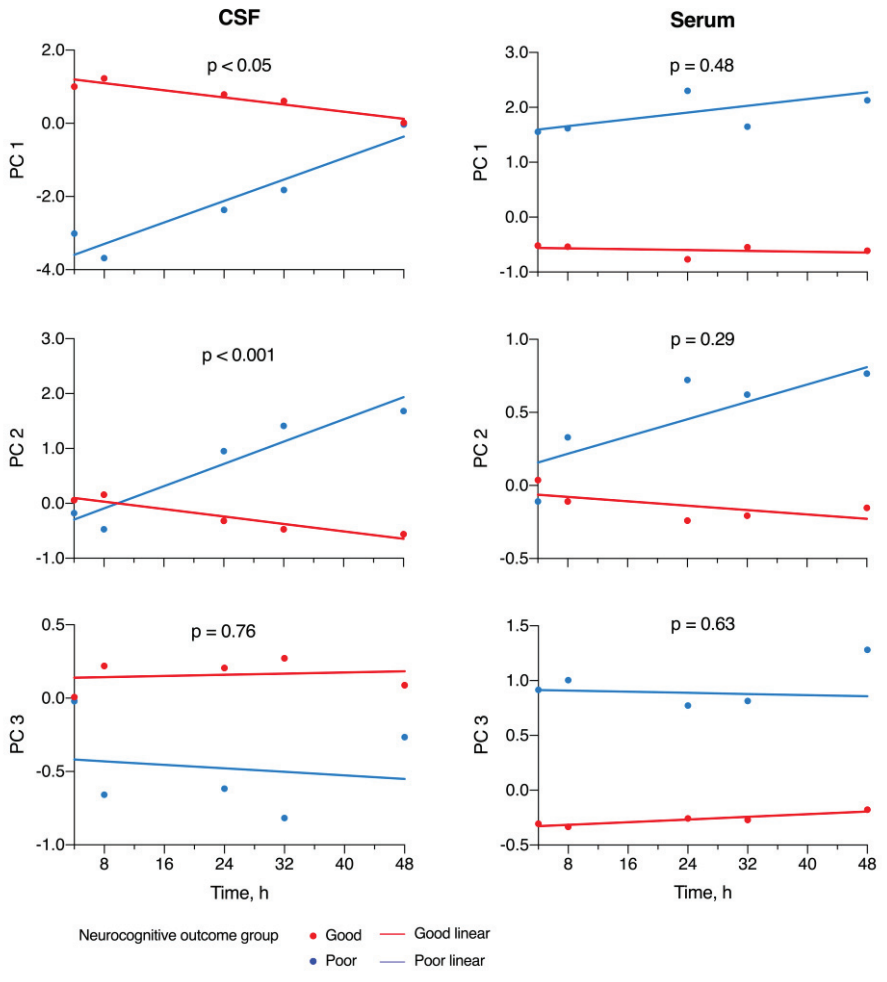

Figure 15. Pre-operative principal components 1-3 based on all 52 inflammatory biomarkers, assessing the group-time interaction in CSF and serum (n=24).Source: Danielson, M. et.al., Ann Neurol, 2020, Creative Commons BY 4.0 License

When individual patient data were applied to the PC2 construct outcome variable, all 6 patients in the Poor neurocognitive outcome group (z-score ≥1.00) showed a gradual increase over time, while this pattern was only seen in 3 out of 18 patients in the Good neurocognitive outcome group (100% vs 17%; p=0.006) (Figure 16).

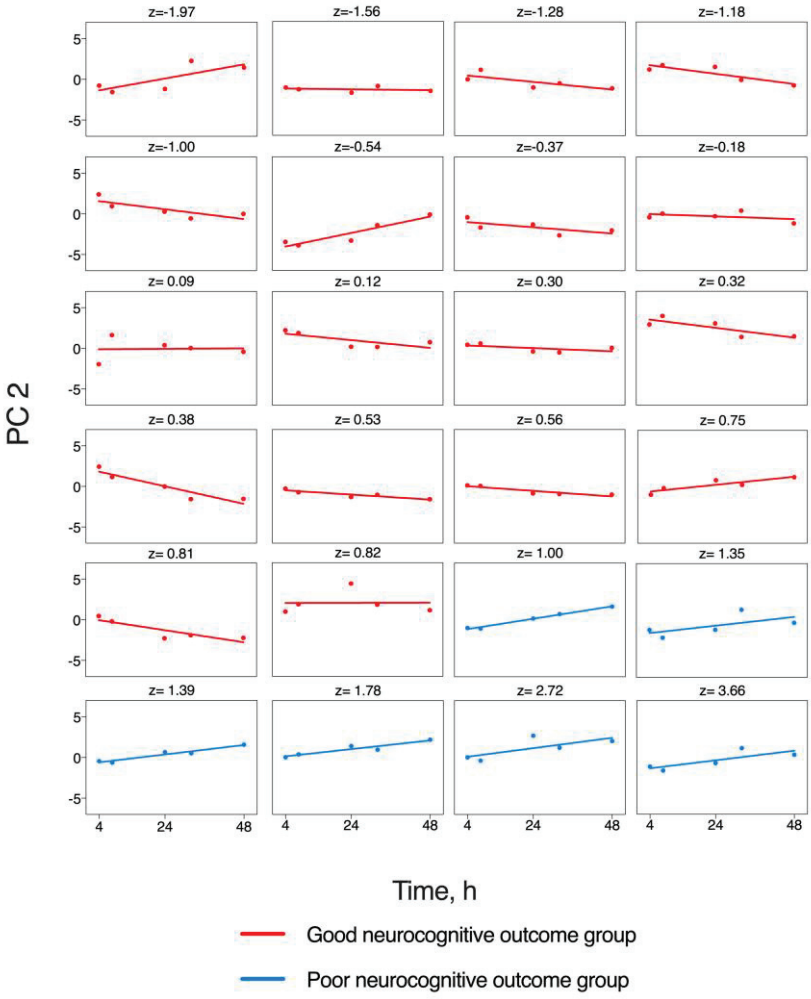

Figure 16. Individual trajectories for the second principal component (PC2) based on all 52 CSF biomarkers (n=24). Individual regressions show that all patients with long-term cognitive decline (blue, n = 6) exhibit an increased CSF inflammatory trajectory over time, as compared to only 3 of 18 patients without long-term cognitive decline (red, n=18) (p=0.006; z indicates individual z-score) Source: Danielson, M. et al., Ann Neurol, 2020, Creative Commons BY 4.0 License

In the serum, none of the three PC construct variables were significantly associated with neurocognitive outcome (p=0.48, p=0.29 and p=0.63 for PC1, PC2 and PC3, respectively) (Figure 15).

The cytokines and chemokines with the highest loadings (impact) on PC2 are shown in Figure 17. A common pattern emerges with separation of the groups at the end of the observation period, with relatively more pronounced expression (inflammation) in the patients who subsequently experienced a poor neurocognitive outcome.

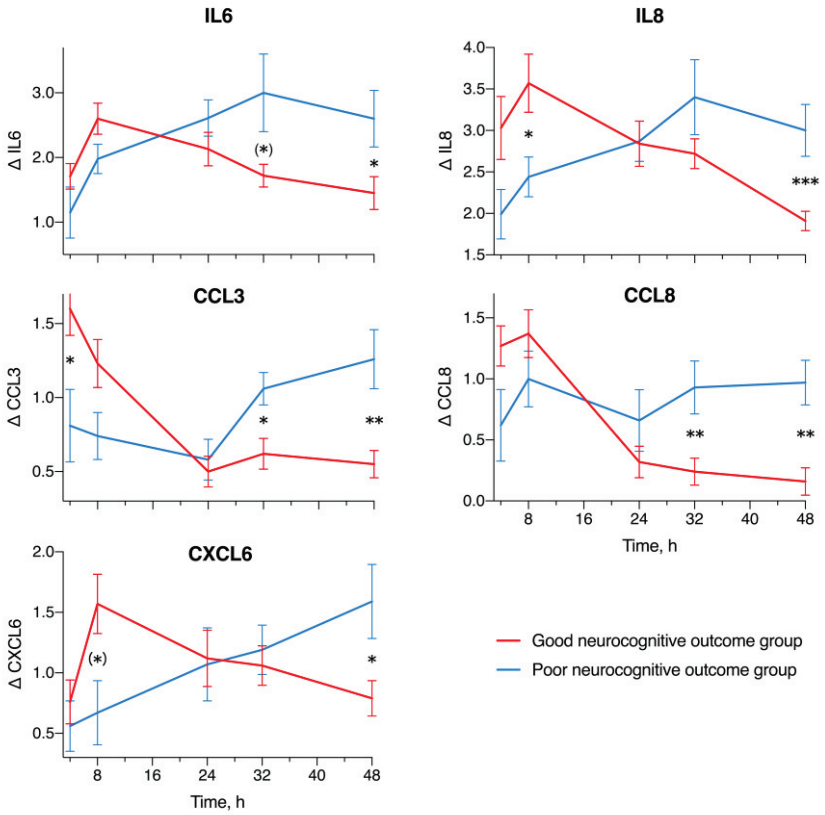

*Figure 17. Postoperative changes in the CSF levels of two cytokines (IL6 and IL8) and three chemokines (CCL3, CCL8 and CXCL6) with respect to neurocognitive outcome (n=24). *p<0.05, **p<0.01, (*)p<0.1. Data are presented as mean ± standard error of the mean. Source: Danielson, M. et al., Ann Neurol, 2020, Creative Commons BY 4.0 License*

4.7.2 Neuroinflammation in cardiac surgery and effects of methylprednisolone (Paper III)

In **Paper III**, the serum levels of IL-6 and IL-8 increased markedly in the control group, a response that was significantly lower in the methylprednisolone group ($p<0.001$) (Figure 19 and Figure 18). The serum level of TNF-α was unaffected in the control group and reduced in the treatment group, a statistically significant difference ($p<0.001$).

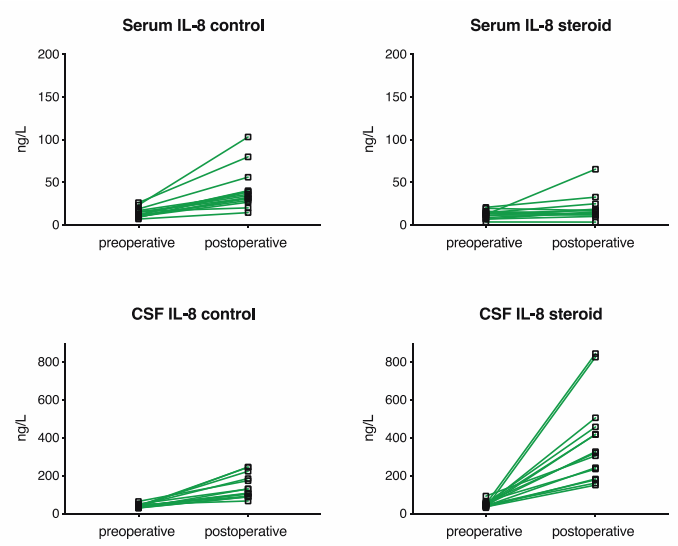

Figure 18. Individual data on the pre- and postoperative levels of serum and CSF IL-8 levels in the steroid-treated and control groups. Source: Danielson, M. et al., J Neuroinflammation, 2018, Creative Commons BY 4.0 License

In the CSF samples, we found increased IL-6 levels after cardiac surgery in the control group, and this increase was significantly attenuated by methylprednisolone ($p=0.001$) (Figure 19). In contrast, the post-operative increase in CSF IL-8 seen in the control group was enhanced in the treatment group ($p<0.001$) (Figure 18). There was a post-operative increase in the CSF level of TNF-α in both groups, with no significant between-group difference.

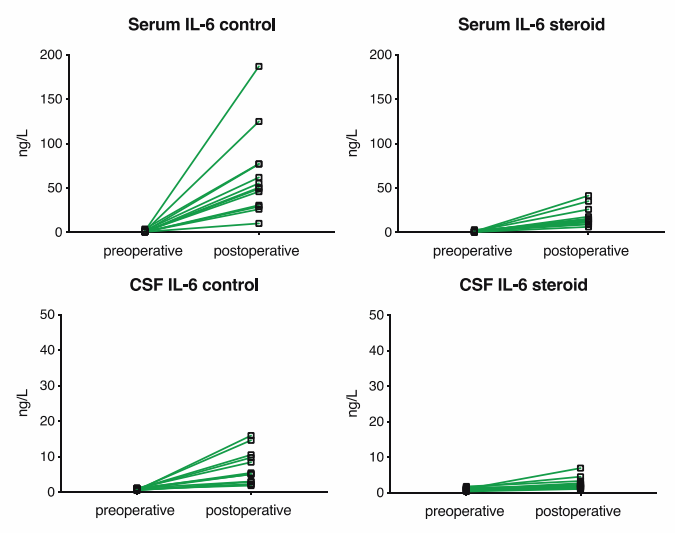

Figure 19. Individual data on the pre- and postoperative levels of serum and CSF IL-6 levels in the steroid-treated and control groups. Source: Paper III.

5. Discussion

This thesis aimed to explore different aspects of the secondary responses to surgery, particularly those involving the CNS. To this end, we have analyzed several biomarkers in the blood and CSF, and using a battery of neuropsychological tests, followed the cognitive trajectories of the patients after surgery.

The studies in this thesis, although by no means without certain limitations (see Section 5.1), each explore some unique aspects of the subject matter. For the first time, the post-operative biomarker changes in the CNS and their relationship to long-term neurocognitive decline have been studied in detail. Also for the first time, the postoperative effects on BBB function and CNS biomarkers of glucocorticoid treatment have been studied.

5.1 Methodological considerations

Orthopedic surgery:
In **Papers I** and **II**, the study population consists of patients who were scheduled for elective total hip or knee arthroplasty. As our focus was on the mechanisms behind the development of POCD, we excluded patients with existing severe mental and cognitive disorders, e.g., preoperative cognitive impairment (MMSE score <24), neurovascular disease, alcohol or drug abuse or other preexisting neurologic or psychiatric disease. To avoid confounding factors when analyzing the inflammatory markers, patients with recent or ongoing treatment with anti-inflammatory drugs were excluded, as were patients with any autoimmune disease. Any contraindication to spinal catheter insertion (coagulopathy, treatment with potent antithrombotic drugs or spinal nerve disorders) was a disqualifying factor.

These rather strict inclusion criteria left us with a comparatively healthy patient study group, which we deemed necessary to maximize the chance of achieving interpretable results. Nevertheless, one should be aware of the risk that the obtained results are less generalizable. In the end, there were no differences between the two outcome groups with respect to demographic data. Compared with data from the Swedish national registries for knee and hip arthroplasty for the period 2014–2016, with a mean age of 68 years and 43% male patients, it appears that the mean age of 70 years in our cohort is similar, whereas the distribution of gender is somewhat skewed with 33% male patients.

A major limitation of the present study is the small sample size, with only six patients in the Poor neurocognitive outcome group. This was to be expected, though, as the frequency of long-term POCD (22%) was similar to that reported in other studies. The definition of POCD varies across studies. We classified patients according to a composite z-score >1, which corresponds to a deterioration from the baseline level of >1 SD, thus including patients with both mild and severe cognitive decline.

A single administration of bupivacaine and sufentanil *via* the intrathecal catheter was used for spinal anesthesia. Bupivacaine has been shown to have anti-inflammatory properties[79, 80] and has also been associated with chemical meningitis[81, 82]. The potential effects of single-dose intrathecal sufentanil or perioperative intravenous sedation with propofol on CSF inflammatory biomarkers are unknown. Another possibility is a localized inflammatory response as a result of the indwelling catheter, and this cannot be excluded from our data. As the anesthetic regimen for the two groups was uniform, it is unlikely that any differences in brain immune responses or signs of neuronal damage between the Good and Poor neurocognitive outcome groups can be attributed to inter-individual differences in the anesthetic management or as a result of the indwelling catheter.

We examined the cognitive function at discharge from the hospital (3–5 days postsurgery) and at 3 months postoperatively. Since early testing may be compounded by hospitalization, pain and the remnants of anesthetic and analgesic drugs, testing for POCD at 3 months postoperatively is thought to provide a more reliable index of cognitive decline[83].

One could argue that the cognitive tests should have been administered by a neuropsychologist, ideally with the same one performing all the tests. We were three investigators who administered the tests at the two test sites. Investigators were previously trained by the group responsible for development of the ISPOCD test battery and the test manual was strictly followed.

Six patients (22%) received 15–30 mg subcutaneous ketorolac, a non-steroidal anti-inflammatory drug, intraoperatively as part of the postoperative pain regimen at one center. The extent to which this might have influenced the levels of CNS or serum inflammatory markers is not known, although the use of ketorolac was evenly distributed between the Good (24%) and Poor (17%) neurocognitive outcome groups.

Cardiac surgery:

In **Paper III**, the study population consisted of 30 patients scheduled for elective surgical aortic valve repair due to aortic stenosis. Overall, 30% of the patients had coronary vessel disease and underwent concurrent CABG. Contraindications to lumbar puncture, recent or ongoing treatment with thrombolytic or potent anti-platelet

drugs or abnormal preoperative or postoperative coagulation tests were excluding criteria, as were severe organ dysfunction, history of neurologic disease and treatment with steroids or other anti-inflammatory drugs.

To minimize the risk of systematic error, a randomized block design based on gender and type of surgery was used. The majority of the participants were male (77%), reflecting the population of patients undergoing cardiac surgery. 2013-2017, 64.6% of the 1130 patients undergoing surgical aortic valve replacement due to aortic stenosis at Sahlgrenska University Hospital, were male.

A lumbar puncture was performed the day before and approximately 24 hours after surgery. This postoperative time-point was chosen to allow for the detection of a need for a re-operation, which might include a second period of extracorporeal circulation (ECC) with the concurrent heparin treatment, as well as clinical and laboratory evaluations of coagulation status. A single postoperative CSF measurement obviously means that early transient changes could be missed, as one might suspect is the case for S-100B, when the graphs in **Papers I** and **III** (Figure 10) are compared.

5.2 Ethical issues

All studies were approved by the Regional Institutional Review Boards. All patients were given written and oral information about the studies and opportunities for questions and discussions.

Patients in **Papers I** and **II**, who would normally have had their single-shot spinal anesthesia delivered via a 25-27G spinal needle, instead had a lumbar intrathecal catheter inserted using an 18G Tuohy needle. The catheter were left in place for 48 hours. The larger diameter needle carries a higher risk of post-dural puncture headache[84]. While leaving a catheter in place reduces this risk[85], the risk probably remains higher than after a conventional spinal anesthesia. One patient in the study was diagnosed with post-dural puncture headache, which was successfully treated with an epidural blood patch.

There is always a small risk for epidural bleeding, with potentially disabling consequences, in connection with a lumbar puncture. To minimize the risk, we used thin, less-trauma-inducing needles and repeated evaluation of coagulation tests in **Paper III**.

A high, perioperative, single dose of corticosteroids has several potential benefits in relation to cardiac surgery and is a part of the standard anesthetic regimen in many centers, although it also has some important potential disadvantages. Mean and peak blood-glucose levels rise, and this has been associated with increased postoperative morbidity and mortality[86]. However, in the present study, plasma glucose levels

were maintained at acceptable levels with insulin infusion in the corticosteroid treatment group. Furthermore, it has been suggested that the use of corticosteroids is associated with higher lactate levels, higher sensitivity to infectious agents, impaired wound healing, and prolonged ventilation time[87, 88]. However, the incidence of infections or the length of ICU or hospital stay did not differ between the groups in **Paper III**.

5.3 Neuroinflammation and cognitive function after major surgery (Paper I)

We explored the material for pre-, peri-, and post-operative differences between patients in the Good and Poor neurocognitive outcome groups to look for clues as to the underlying mechanism(s) that would explain the observed differences in neurocognitive outcome. There were no differences in demographics or intraoperative characteristics between the groups with poor and good neurocognitive outcomes (Table 1). Although we found no associations between the preoperative levels of CSF or blood biomarkers and postoperative cognitive outcome, this could be due to the small sample size. A meta-analysis conducted in 2013 found a correlation between POCD and higher preoperative levels of serum IL-10 and S-100B[89].

In line with the results of previous studies, we found a pronounced increase in the levels of systemic inflammatory biomarkers following surgery. In animal studies this has been shown to disrupt the BBB and induce a neuroinflammatory response[32].The permeability of the BBB (measured by the CSF-albumin to serum-albumin ratio) was significantly increased after surgery (Figure 9). The CSF levels of the astrocyte cell injury markers GFAP and S-100B increased significantly postoperatively (Figure 10). The overall CNS and blood inflammatory changes were explored using a principal component analysis (PCA). Between the neurocognitive outcome groups, there were no significant differences in the trajectories of the blood inflammatory biomarkers, the CSF astrocyte injury markers, or the BBB permeability changes.

This brings us to the major finding in **Paper I**, which is that patients with long-term postoperative cognitive decline show a differential neuroinflammatory response trajectory, as compared to patients with good cognitive outcomes. Patients with a good neurocognitive recovery have an initial relative increase in CSF inflammatory biomarkers, followed by a gradual decline. This is in contrast to patients with a poor neurocognitive recovery, who have a lower initial expression of inflammatory biomarkers, followed by a continuous increase during the 48-hour study period (Figure 15). Following this gateway analysis, a more detailed analysis of the biomarkers with

the highest PC loadings revealed a smaller set of CNS chemokines and cytokines that showed significant differences between the patients with good and poor neurocognitive outcomes (Figure 17). The recurring pattern is one of significantly increased late inflammatory responses in patients with long-term cognitive decline.

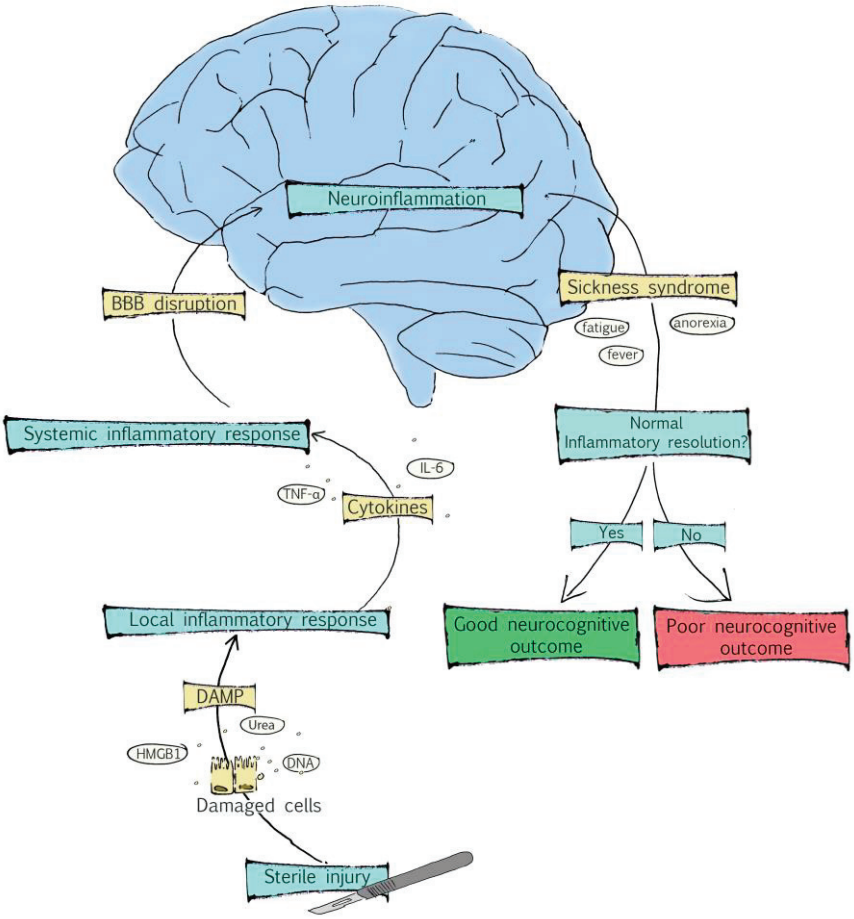

Figure 20. A neuroinflammatory theory of postoperative cognitive decline.

These findings suggest that systemic inflammation and BBB disruption are natural responses to major surgery but not a cause of POCD *per se*. With disruption of the BBB, a neuroinflammatory secondary response in this setting is probably inevitable, and it seems that a difference in inflammatory cascade timing and resolution may be the key to understanding the mechanisms behind long-term neurocognitive decline after a surgical trauma (Figure 20). During the latter stage of our sampling period, the

cerebral inflammatory activity in the Poor neurocognitive outcome group was steadily increasing (Figure 15). However, what events occur after the 48 hours remain unclear. It is, however, interesting to note that in the previously mentioned PET study of Forsberg et al., in which postoperative neuroinflammation was detected using a radioligand that is selective for the translocator protein expressed by microglia[63], a late (at 3 months) upregulation of cerebral immune activity was correlated to POCD. A suppressive response on Days 3–4 was followed by microglial activation at 3 months in the subset of patients with cognitive decline.

While there was a strong association between CNS inflammatory trajectories and poor long-term cognitive outcome, we were unable to detect any association to the short-term (3–5 days) postoperative cognitive status, suggesting that other factors, such as persisting drug effects, pain and lack of sleep, are of greater importance when it comes to short-term POCD. This is in line with the findings of Hirsch and colleagues who, in a small study of 10 patients, found no associations between blood and CSF inflammatory mediator levels during the first 18 hours after surgery and early (1–3 days) POCD[62].

5.4 Neuronal injury and cognitive function after major surgery (Paper II)

This secondary analysis of the data collected in the NEUPORT study explores temporal changes in markers of neuronal injury and brain amyloidosis and possible associations with postoperative neurocognitive outcomes at hospital discharge and at 3 months after major orthopedic surgery.

The main findings of this study are that CSF and systemic levels of the neuronal injury markers T-tau and NSE (Figure 11, Figure 13), as well as the brain amyloidosis marker Aβ1-42 (Figure 14) increase post-surgery, suggesting that major orthopedic surgery potentially induces neuronal stress or damage. However, as there were no differences in the levels of release of these neuronal injury markers or Aβ1-42 between patients who developed adverse postoperative neurocognitive outcome or did not, it seems unlikely that adverse neurocognitive outcomes after surgery are associated with surgery-induced neuronal injury or release of the AD marker Aβ1-42. The postoperative levels of the plasma to CSF ratios of T-tau, and Aβ1-42 were higher in the poor neurocognitive outcome group, suggesting a greater perturbation of the BBB in this outcome group.

Evered and coworkers studied changes in neuronal injury markers in patients undergoing joint arthroplasty under general anesthesia [30] and found a gradual increase in the plasma level of NFL over 48 hours, while plasma T-tau peaked after 6 hours. In the present study, we can confirm this pattern of systemic release of neuronal

injury markers. However, in contrast to the study of Evered et al., our patients were operated under regional anesthesia, suggesting that the primary impact on the CNS is related to the surgical trauma rather than to general anesthesia. Furthermore, Evered and coworkers did not present data on the CSF levels of neuronal injury markers and they did not measure late neurocognitive outcome in their study, and therefore could not relate surgery-induced changes in plasma levels of neuronal injury markers to late cognitive function.

The CSF levels of T-tau increased gradually during the 48 hours after the surgical procedure, while the plasma levels of T-tau showed an early peak (4 hours), corresponding to the timing of the opening of the BBB (Figure 11). In contrast, we were unable to detect any significant changes in the CSF levels of NFL within the study period. However, NFL is known to have a different time-course, with a serum half-life of several weeks[69]. Moreover, following a stroke, the levels of NFL in CSF and serum increase continuously during the first 3 weeks[70]. Thus, our failure to capture changes in the CSF levels of NFL could be due to a protracted increase in CSF-NFL that become apparent after the 48-hour sampling period. The increase in serum-NFL could be explained by the change in BBB permeability, combined with the positive CNS-to-serum gradient for NFL. Alternatively, the gradual increase in serum-NFL could be associated with surgery-induced peripheral nerve injury. There was an absent (T-tau) and poor (NFL) correlation between blood and CSF levels of these markers, suggesting that one should be cautious when evaluating surgery-induced neuronal injury that solely rely on blood sampling.

Both CSF-NSE and serum-NSE showed significant changes over time (Figure 13). Considering that there was a prominent negative CSF-to-serum gradient (higher concentrations in serum) and that there is an abundance of extra-cerebral sources of NSE (peripheral neurons, erythrocytes, platelets and neuroendocrine cells), any changes in NSE level, be they in the serum or CSF, must be regarded with caution.

We found a significant and continuous increase in the CSF level of Aβ1-42 during the study period (Figure 14). Perioperative changes in the CSF Aβ1-42 levels are not well-studied. In a study of 11 patients who were undergoing idiopathic nasal CSF leakage correction, Tang et al. measured the CSF levels of AD biomarkers up to 48 hours postoperatively; they found no changes in the CSF levels of Aβ1-42 while the CSF levels of T-tau increased[90]. Nonetheless, increased postoperative levels of CSF Aβ1-42 have been detected 24 hours[42] and 1 week[91] after cardiac surgery. It has been speculated that the neuroinflammatory response to major surgery induces proteolysis and cleavage of the amyloid precursor protein, which would increase the level of Aβ1-42. A hallmark sign of AD is a low CSF level of Aβ1-42, presumably because of sequestration into senile plaques. Low preoperative CSF levels of Aβ1-42 were in one study shown to be a predictor of neurocognitive decline at 3 months, and it has been suggested that patients with subclinical AD neuropathology are at risk of

developing postoperative neurocognitive decline[92]. In our study, we found no differences in the preoperative CSF levels of Aβ1-42 between the Good and Poor neurocognitive outcome groups. When applying the clinically used AD cutoff values for preoperative CSF Aβ1-42, CSF T-tau and the ratio thereof, there was no association with cognitive outcome.

5.5 Prevention of the neuroinflammatory response to major surgery by a corticosteroid (Paper III)

In 2012, Reinsfelt and colleagues reported a pronounced neuroinflammatory response to cardiac surgery coupled with disruption of the BBB function[49]. In a later MRI study, 47% of the patients showed signs of BBB damage after CABG[51]. This could explain the observed development of brain edema after otherwise uncomplicated cardiac surgery[93]. Furthermore, cardiac surgery is coupled with a high risk for perioperative cerebral microembolization, and this has been proposed as a mechanism for increased BBB permeability[94, 95]. In addition, cerebral oxygen supply/demand mismatch has been proposed as a mechanism for cognitive dysfunction after cardiac surgery[94].

Another possible explanation for the impaired BBB function is an effect of the systemic inflammatory reaction triggered by the surgical trauma. Experimental studies have shown that peripheral surgery induces rapid disruption of the BBB *via* proinflammatory cytokines, promoting macrophage migration into the CNS[32]. It is well-known that cardiac surgery triggers a pronounced systemic inflammatory reaction[55]. This is thought to be a consequence of both a major operation and the cardiopulmonary bypass that exposes the blood, and thus the immune system, to a large surface of foreign material during extracorporeal circulation.

In **Paper III**, we wanted to explore further the neurochemical response to cardiac surgery, and whether a perioperative treatment with a potent anti-inflammatory drug would modulate the BBB dysfunction and, thus, the postoperative neuroinflammation. Methylprednisolone is a synthetic corticosteroid with a half-life of 1.7–5.2 hours and negligible mineralocorticoid effects. It has been used to counteract the systemic inflammatory reaction after cardiac surgery[96] in doses of 15–30 mg/kg administered as a single-dose treatment. We found an increased CSF-albumin to serum-albumin ratio (i.e., disruption of the BBB function) in both groups, confirming the results of Reinsfelt et al. [49]. As there was no significant difference between the groups, we concluded that methylprednisolone exerts no protective effect on post-surgical BBB dysfunction.

There was a pronounced postoperative increase in the serum levels of IL-6 and IL-8, corresponding to a systemic inflammatory reaction that predictably was significantly attenuated in the methylprednisolone-treatment group (Figure 18, Figure 19). When it came to evaluating cerebral inflammation, the CSF level of IL-6 showed the same pattern as the serum levels of IL-6. However, as the concentrations were lower in the CSF this might just reflect a spillover from the blood, thus not proving a reduction of neuroinflammation *per se*.

To address the question of an ongoing inflammatory reaction within the CNS we turned to IL-8. From our previous study[49], we know that there are higher concentrations of IL-8 in the CSF, as compared to serum, which would rule out diffusion as an explanation and would instead suggest intrathecal production.

Indeed, the concentration of CSF-IL-8 in the placebo group was higher than the serum-IL-8, with a marked increase postoperatively. Much to our surprise, there was an even more pronounced increase in CSF-IL-8 after surgery in the methylprednisolone group, indicating exacerbated neuroinflammation after corticosteroid treatment. Reports in the literature on the effects of glucocorticoid treatment on CSF-IL-8 production after surgery are few and far between. In a study on post-herpetic neuralgia treatment with intrathecal injection with methylprednisolone, the CSF level of IL-8 was reported to be increased 8-fold[97].

Although there was a small increase in the CSF level of T-tau after surgery in the control group, we found no convincing biochemical evidence for neuronal damage, as neither the CSF-NSE nor CSF-NFL was affected 24 hours after cardiac surgery, supporting the findings of Reinsfelt et al.[49]. There was a small increase in the CSF level of S-100B in the methylprednisolone group, and a small increase in the CSF level of T-tau in the control group. However, there were no significant between-group differences. Kaukinen et al. have shown that normothermic or hypothermic cardiac surgery with CPB does not induce increased levels of CSF-NSE, measured days and months after the procedure[98]. This is in agreement with the results from a previous[49] and the present study, showing that that CSF levels of NSE, NFL and T-tau are not elevated 24 hours after cardiac surgery.

6. Conclusions

I. Major surgery induces a temporary increase in the permeability of the BBB.

II. Major surgery is associated with pronounced increases in both the systemic and CSF levels of inflammatory biomarkers.

III. The neuroinflammatory biomarkers in patients undergoing orthopedic surgery show a strong correlation to long-term postoperative cognitive decline.

IV. Orthopedic surgery causes the release of CSF biomarkers of neural injury and brain amyloidosis, suggesting neuronal damage or stress.

V. The CSF and systemic biomarkers of neuronal injury or brain amyloidosis in patients undergoing orthopedic surgery show no association to long-term postoperative cognitive decline.

VI. None of the preoperatively measured biomarkers of inflammation, neuronal damage or brain amyloidosis are capable of predicting an unfavorable neurocognitive outcome.

VII. A perioperative single-dose methylprednisolone treatment attenuates the systemic inflammatory reaction but does not prevent the postoperative BBB dysfunction or the neuroinflammation seen after cardiac surgery

7. Future perspectives

The long-term goal of POCD research should be to develop preventive measures and treatment options for this common and potentially devastating condition. In order to accomplish this, the underlying mechanisms need to be understood.

In **Paper I** we found a strong association between cerebrospinal inflammatory biomarker trajectories and long-term postoperative neurocognitive outcome. However, the group with poor neurocognitive outcome consisted of mere six patients and these findings should therefore, ideally, be validated in a larger study.

In **Paper II** there was no association between neither systemic nor cerebrospinal markers of neuronal cell damage and postoperative neurocognitive outcome. Although the post-hoc power analysis indicated that the sampling size should be large enough to detect any relevant difference, the negative result could still be due to an underpowered study and thus this should also be addressed in a larger study. Given the delayed and protracted release of NFL, an extended sampling period could give useful data.

In **Paper III** we were unable to prevent the BBB disruption after cardiac surgery in the steroid-treatment group. That cytokine-induced changes in BBB permeability are involved in post-operative neuroinflammation and cognitive impairment has been shown in animal studies[32, 99], and this was prevented when either inhibiting TNF-α activity or stimulating the cholinergic anti-inflammatory pathway with choline or nicotine[32]. In a study of 382 patients undergoing non-cardiac surgery in general anesthesia, a history of tobacco-smoking reduced the risk of POCD after 1 week[100]. Ketamine, an NMDA receptor antagonist, is an anesthetic drug with hypnotic and analgesic properties. Ketamine is believed to exert neuroprotective effects through a reduction of apoptosis, microthrombosis and inflammation[101]. A recent meta-analysis concludes that ketamine treatment seems to reduce the risk of POCD[102].

To evaluate the preventive effects of pre- / peri-operative anti-TNF, nicotinergic and ketamine treatment on BBB function, neuroinflammation and long-term cognitive decline in a larger study seems to me like a worthwhile undertaking.

Acknowledgements

Professor *Sven-Erik Ricksten*, brilliant scientist, enthusiastic teacher and always supportive supervisor. Thank you for taking me under your wing.

Ann Westerlind, co-supervisor, always willing to lend a helping hand when I ran into recruitment issues or encountered clinical pitfalls

Björn Reinsfelt, who guided me when I, on shaky legs, took my first steps onto scientific ground. Dearly, and daily, missed.

Bengt Nellgård, co-supervisor and infallible optimist.

Lars I Eriksson, *Henrik Zetterberg*, *Kaj Blennow*, *Fredrik Granath* and the other *co-authors* from Stockholm and Gothenburg, for a fruitful collaboration

Lukas Lannemyr and *Jonatan Oras* for invaluable statistical advice

Martin Fredholm, long-time friend and globetrotting companion, together we have experienced not only the ups and downs of clinical and academic careers, but also poverty-stricken flea-pits as well as pristine beaches on the other side of the planet.

Ulla Nathorst-Westfelt and *Bengt Redfors*, former and present heads of Cardiothoracic Anesthesia as well as *Helena Rexius*, former head of the Department of Cardiothoracic Surgery who let me set aside the time needed from clinical work.

All my colleagues and friends at Thoraxoperation and TIVA. The *anesthesiologists*, never hesitating to cover for me when I hastily went for another bike-ride to Mölndal in my pursuit of recruiting patients. The surgical and ICU teams; *nurses*, *assistant nurses*, *perfusionists*, and *surgeons*. Working with you is a true privilege.

A special mention goes to *Klaus Kirnö*, clinical wizard and informal mentor, and to *Carl-Johan Malm,* who eventually excavated demographic data from the heart-surgery registry.

David, for occasional distractions and reality-checks, even though you referred to this endeavor as 'flogging a dead horse'…

Flaggan, *Eric*, *Jonas* and the rest of the *Bored Game-Geeks* for things unscientific.

Parents, *brother* & *sisters*. Göran, Liselotte, Arne, Margareta, Katarina, Jonas, Kristin. Giving me support in matters small and large, and, I know, mourning the absence of post-dissertation festivitas in these corona-ridden times..

Liv, *Isak* and *Anna*, for being everything to me.

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Appendix

Appendix table 1. List of all 92 inflammatory CSF and blood-borne biomarkers

Cytokine related	CSF1	Macrophage colony-stimulating factor 1
Cytokine related	FLT3LG	Fms-related tyrosine kinase 3 ligand
Cytokine related	IFNG	Interferon gamma
Cytokine related	IL10	Interleukin-10
Cytokine related	IL10RA	Interleukin-10 receptor subunit alpha
Cytokine related	IL10RB	Interleukin-10 receptor subunit beta
Cytokine related	IL12B	Interleukin-12 subunit beta
Cytokine related	IL13	Interleukin-13
Cytokine related	IL15RA	Interleukin-15 receptor subunit alpha
Cytokine related	IL17A	Interleukin-17A
Cytokine related	IL17C	Interleukin-17C
Cytokine related	IL18	Interleukin-18
Cytokine related	IL18R1	Interleukin-18 receptor 1
Cytokine related	IL1A	Interleukin-1A
Cytokine related	IL2	Interleukin-2
Cytokine related	IL20	Interleukin-20
Cytokine related	IL20RA	Interleukin-20 receptor subunit alpha
Cytokine related	IL22RA1	Interleukin-22 receptor subunit alpha-1
Cytokine related	IL24	Interleukin-24
Cytokine related	IL2RB	Interleukin-2 receptor subunit beta
Cytokine related	IL33	Interleukin-33
Cytokine related	IL4	Interleukin-4
Cytokine related	IL5	Interleukin-5
Cytokine related	IL6	Interleukin-6
Cytokine related	IL7	Interleukin-7
Cytokine related	IL8	Interleukin-8
Cytokine related	LIF	Leukemia inhibitory factor
Cytokine related	LIFR	Leukemia inhibitory factor receptor
Cytokine related	LTA	Lymphotoxin-alpha
Cytokine related	OSM	Oncostatin-M
Cytokine related	SLAMF1	Signaling lymphocytic activation molecule
Cytokine related	STAMBP	STAM-binding protein
Cytokine related	TGFB1	Transforming Growth Factor β1
Cytokine related	TNF	Tumor necrosis factor
Cytokine related	TNFRSF11B	Tumor necrosis factor receptor superfamily member 11b
Cytokine related	TNFRSF9	Tumor necrosis factor receptor superfamily member 9
Cytokine related	TNFSF10	Tumor necrosis factor superfamily member 10
Cytokine related	TNFSF11	Tumor necrosis factor superfamily member 11
Cytokine related	TNFSF12	Tumor necrosis factor superfamily member 12
Cytokine related	TNFSF14	Tumor necrosis factor ligand superfamily member 14
Cytokine related	TSLP	Thymic stromal lymphopoietin

Chemokine related	CCL11	C-C motif chemokine 11
Chemokine related	CCL13	C-C motif chemokine 13
Chemokine related	CCL19	C-C motif chemokine 19
Chemokine related	CCL2	C-C motif chemokine 2
Chemokine related	CCL20	C-C motif chemokine 20
Chemokine related	CCL23	C-C motif chemokine 23
Chemokine related	CCL25	C-C motif chemokine 25
Chemokine related	CCL28	C-C motif chemokine 28
Chemokine related	CCL3	C-C motif chemokine 3
Chemokine related	CCL4	C-C motif chemokine 4
Chemokine related	CCL7	C-C motif chemokine 7
Chemokine related	CCL8	C-C motif chemokine 8
Chemokine related	CX3CL1	Fractalkine
Chemokine related	CXCL1	C-X-C motif chemokine 1
Chemokine related	CXCL10	C-X-C motif chemokine 10
Chemokine related	CXCL11	C-X-C motif chemokine 11
Chemokine related	CXCL5	C-X-C motif chemokine 5
Chemokine related	CXCL6	C-X-C motif chemokine 6
Chemokine related	CXCL9	C-X-C motif chemokine 9
Chemokine related	KITLG	KIT Ligand
Leukocyte cell surface	CD244	Natural killer cell receptor 2B4
Leukocyte cell surface	CD274	Programmed cell death 1 ligand 1
Leukocyte cell surface	CD40	CD40L receptor
Leukocyte cell surface	CD5	T-cell surface glycoprotein CD5
Leukocyte cell surface	CD6	T-cell surface glycoprotein CD6 isoform
Neurotrophic growth factors	ARTN	Artemin
Neurotrophic growth factors	BDNF	Brain-derived neurotrophic factor
Neurotrophic growth factors	FGF19	Fibroblast growth factor 19
Neurotrophic growth factors	FGF21	Fibroblast growth factor 21
Neurotrophic growth factors	FGF23	Fibroblast growth factor 23
Neurotrophic growth factors	FGF5	Fibroblast growth factor 5
Neurotrophic growth factors	GDNF	Glial cell line-derived neurotrophic factor
Neurotrophic growth factors	HGF	Hepatocyte growth factor
Neurotrophic growth factors	NGF	Beta-nerve growth factor
Neurotrophic growth factors	NRTN	Neurturin
Neurotrophic growth factors	NTF3	Neurotrophin-3
Neurotrophic growth factors	TGFA	Transforming growth factor α
Neurotrophic growth factors	VEGFA	Vascular endothelial growth factor A
Miscellaneous	ADA	Adenosine Deaminase
Miscellaneous	AXIN1	Axin-1
Miscellaneous	CASP8	Caspase 8
Miscellaneous	CDCP1	CUB domain-containing protein 1
Miscellaneous	CST5	Cystatin D
Miscellaneous	DNER	Delta and Notch-like epidermal GF-related recep
Miscellaneous	EIF4EBP1	Eukaryotic translation initiation factor 4E-BP1
Miscellaneous	MMP1	Monocyte chemotactic protein 1
Miscellaneous	MMP10	Matrix metalloproteinase 10
Miscellaneous	PLAU	Plasminogen activator, urokinase
Miscellaneous	S100A12	S100 calcium-binding protein A12
Miscellaneous	SIRT2	SIR2-like protein 2
Miscellaneous	SULT1A1	Sulfotransferase family 1A member 1